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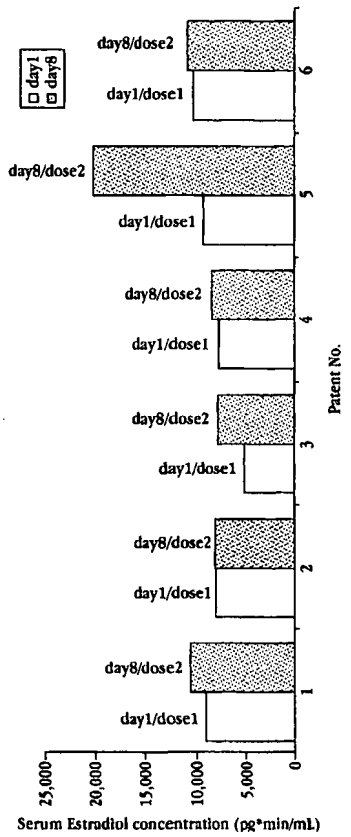
(54) Title: METHODS AND COMPOSITIONS FOR TREATING BENIGN GYNECOLOGICAL DISORDERS, CONTRACEPTION, AND HORMONE REPLACEMENT



(57) Abstract: A method and improvement of treating benign gynecological disorders is described. The method is also suitable for contraception and for hormone replacement therapy. In the method, treatment of a benign gynecological disorder with a composition comprised of a gonadotropin releasing hormone (GnRH) compound and an estrogenic compound, and optionally, an androgenic compound. The composition may be formulated as a nasal spray comprised of a GnRH compound and an estrogenic compound in the form of a water-soluble complex with a water-soluble cyclodextrin. The present invention further relates to an improvement in a method of contraception, in treatment of benign gynecological disorders, and in hormone replacement by administering intranasally an estrogenic compound and an androgenic compound, and in some embodiments an optional progestin compound, in a once-daily bolus formulation comprised of the two or three steroids complexed with a cyclodextrin. An intranasal delivery system for administration of the formulation is also described.

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METHODS AND COMPOSITIONS FOR TREATING BENIGN GYNECOLOGICAL DISORDERS, CONTRACEPTION, AND HORMONE REPLACEMENT

Field of the Invention

[0001] The present invention relates to compositions and methods for treating benign gynecological disorders and/or for contraception by administration of a composition comprised of a gonadotropin releasing hormone (GnRH) compound and an estrogenic compound, and optionally, an androgenic compound. The present invention further relates to an improvement in hormone replacement for postmenopausal or surgically postmenopausal women where an estrogenic compound and an androgenic compound and, optionally, a progestin compound are administered. The improvement involves administering an estrogenic compound and an androgenic compound and, optionally, a progestin compound intranasally in a once-daily bolus of a formulation comprised of the two or three steroids complexed with a cyclodextrin.The invention also relates to methods for preparing the compositions and to methods of treatment using such compositions. The composition is useful for mucosal delivery, particularly nasal delivery.

Background of the Invention

[0002] During a woman's reproductive life, a delicate and complex interplay of hormones are timed and controlled by the hypothalamus. The hormones that participate in the feedback system regulating the menstrual cycle include estrogens and progesterone, the pituitary gonadotropins FSH (follicle stimulating hormone) and LH (luteinizing hormone), and gonadotropin-releasing hormone (GnRH) from the hypothalamus.

[0003] The menstrual cycle is usually divided into a follicular or proliferative phase and a luteal or secretory phase. The length of a normal menstrual cycle is defined as the time from the onset of one menstrual bleeding episode to the onset of the next. Towards the end of one menstrual cycle, plasma levels of estrogen and progesterone fall. Approximately a week prior to ovulation, estradiol levels begin to rise. Just prior to ovulation, estradiol secretion reaches a peak and then falls before rising again after ovulation. Plasma progesterone begins to rise just prior to midcycle and reaches its peak during the luteal phase.

[0004] During a women's reproductive years, defined as the time between onset of menses (menarche) and the final episode of menstrual bleeding (menopause), that is a

premenopausal woman, a variety of benign gynecological disorders can occur. Common benign gynecological disorders include, but are not limited to, premenstrual syndrome, endometriosis, uterine leiomyomata (uterine fibroids), and polycystic ovarian syndrome. Administration of one or more of the hormones involved in regulation of the menstrual cycle has been proposed for relief of the symptoms associated with the disorder or for treatment of the symptoms related to the disorder.

[0005] For example, hormonal therapy has been contemplated for management of premenstrual syndrome, including its most severe form, namely late luteal phase dysphoric disorder. The essential feature of premenstrual syndrome is a pattern of clinically significant emotional and behavioral symptoms that occur during the last week of the luteal phase and remit within a few days after the onset of menstruation. In most females, these symptoms occur in the week before and remit within a few days after the onset of menses. Non-menstruating females who have had a hysterectomy but retain ovarian function may also report similar symptoms. Commonly experienced symptoms of premenstrual syndrome include marked affective lability (*e.g.*, sudden episodes of sadness or irritability), persistent feelings of irritability, anger or tension, feelings of depression and self-deprecating thoughts, decreased interest in usual activities, fatigue and loss of energy, a subjective sense of difficulty in concentrating, changes in appetite, cravings for specific foods, sleep disturbance, breast tenderness or swelling, headaches, joint or muscle pain, a sensation of bloating, and weight gain. The symptoms are often so severe as to seriously interfere with work or with usual social activities or relationships with others.

[0006] Hormonal therapy is one approach to treating benign gynecological disorders. In particular, therapy with a compound that inhibits or suppresses gonadotropin releasing hormone (GnRH) has been proposed (U.S. Patent Nos. 5,340,584; 5,340,585; 5,681,817). GnRH, also known as luteinizing hormone releasing hormone (LHRH), is produced by the hypothalamus, as noted above. Synthetic agonists and antagonists of GnRH administered to premenopausal women have been shown to produce a sustained suppression of FSH/LH release after, in the case of agonists, a transient rise in FSH/LH. Thus, both GnRH agonists and GnRH antagonists are able to reduce serum estradiol and serum progesterone levels. However, a reduced level of the sex hormones is often accompanied by side effects including hot flashes, fatigue, headache, depression, decreased libido, and most significantly, loss of bone mineral density.

[0007] Because of these side effects, hormonal therapies utilizing a GnRH compound typically include administration of an estrogen compound and/or a progestin compound; so-

called "add-back" hormonal therapy. The progestin compound is administered for some portion of each month to induce shedding of the endometrial lining or continuously in order to protect the female from endometrial hyperplasia. For example, in U.S. Patent No. 5,340,585 administration of a GnRH compound for treating benign gynecological disorders is described. The GnRH compound is co-administered with an estrogenic compound to minimize the side effects that result from the reduction in estradiol levels by the GnRH compound. The composition, however, is specifically limited to use in women in whom the risk of endometrial stimulation is minimized or absent, such as women who have had a hysterectomy, those using a progesterone releasing intrauterine device, or those taking a separate progestin.

[0008] Uterine leiomyomata (uterine fibroids) is a common disorder, with one in four women affected at some point in her reproductive life (HARRISON'S PRINCIPLES OF INTERNAL MEDICINE, 12th Ed. Wilson, J. *et al.* Eds., McGraw-Hill, New York, 1991). Many of the women with leiomyoma are asymptomatic and the diagnosis is made during a routine pelvic examination. The condition, however, can be associated with excessive menstrual bleeding or significant pelvic pain.

[0009] Endometriosis is another benign disorder characterized by the presence and proliferation of tissue resembling endometrium outside the endometrial cavity. The disorder is frequently associated with pelvic pain.

[0010] Polycystic ovarian disease is another benign disorder that is characterized by chronic anovulation, infertility, hirsutism, obesity, and amenorrhea or oligomenorrhea.

[0011] Another example of hormonal therapy based on administration of a GnRH agonist has been proposed to ameliorate symptoms associated with premenstrual syndrome (Mortola, J. F., *et al., J. Clin. Endocrin. Metab.*, 72:252A-252F (1991)). The therapy includes co-administration of an estrogen and co-administration for a portion of the 28 day treatment period, a progestin, medroxyprogesterone acetate.

[0012] The prior art also reports parenteral administration of GnRH compounds with co-administration of an oral estrogen (Sugimoto, A. *et al.*, *Fertility and Sterility*, 60(4):672 (1993)). A progestin, medroxyprogesterone acetate, was added for 14 days of each calendar month for endometrial protection in hirsute women, but was omitted in other test patients to prevent disease-specific symptoms associated with progestins. In the patients receiving unopposed estrogen, that is in the women treated with a GnRH and estrogen in the absence of a progestin, 4 of the 12 females had simple endometrial hyperplasia.

[0013] Manipulation of the hormonal balance is also a recognized approach of

contraception. In particular, administration of a GnRH compound for contraception has been described (U.S. Patent Nos. 5,340,584; 5,211,952). Typically, the GnRH compound is administered in a slow or controlled-release fashion for continuous suppression of ovarian estrogen and progesterone production, which suppresses ovarian follicle development and sex steroid production. Estrogen, often a progestin, and sometimes an androgen, are "added-back" to ameliorate the effects of hormonal deficiency. The hormone add-backs are also often administered in a slow, controlled-release or time-release fashion to maintain a constant hormonal serum level.

[0014] A woman's endogenous level of estrogen is significantly reduced upon entering menopause or upon premature surgical menopause induced by removal of the ovaries. The amount of a woman's endogenous estrogen is typically reduced to less than about 10% to about 20% of premenopausal levels following natural or surgical menopause. This reduction of endogenous estrogen levels results in the loss of estrogen's health protective effects, particularly with respect to bone mineral density. Estrogen replacement therapy (ERT) is often utilized as a treatment to increase the level of estrogen in women having reduced levels of endogenous estrogen resulting from natural or surgical menopause. Supplemental estrogen is provided to the women in order to inhibit, ameliorate, or prevent diseases or conditions which result from the reduction of endogenous estrogen.

[0015] The administration of drugs by absorption through mucosae, such as the nasal mucosa or vaginal mucosa, has been of considerable interest in recent years. This route of drug delivery is an alternative to oral administration in cases where drugs are poorly absorbed or are extensively metabolized in the gastrointestinal tract or subjected to first-pass metabolism in the liver. In particular, nasal mucosa has the desirable properties of being highly vascular leading to rapid uptake and the avoidance of first-pass metabolism in the liver, since the venous system from the nose passes directly into the systemic circulatory system. The nasal mucosa also exhibits moderate permeability to water-soluble compounds, comparable to that of the ileum. The permeability of nasal mucosa is higher for most compounds than other mucosae, due in part to the difference in structure of the cells lining the body cavities.

[0016] Efficiency of delivery of drugs by an intra-nasal route is influenced by the degree and rapidity of enzymatic degradation, the nasal clearance rate, as well as the drug's permeability through the mucosa. The clearance rate is produced by the coordinated movement of cilia and is known to be highly dependent upon the prevailing physiological and pathological conditions. Thus, for many drugs administration intranasally is inefficient

due to low uptake of the drug, hence low bio-availability.

[0017] Another potential problem associated with intranasal delivery is mucosal irritation. Irritation caused by the drug itself and/or by absorption or penetration promoters or enhancers often limits the success of nasal formulations. Chronic administration of irritating nasal formulations can cause necrosis, inflammation, exudation, removal of the epithelial monolayer or can lead to irreversible damage to the nasal mucosa.

[0018] Nasal formulations comprised of a GnRH compound have been described (see, for example, U.S. Patent Nos. 5,116,817; 4, 476,116). Nasal formulations for delivery of female sex hormones have been described (see, for example, U.S. Patent Nos. 4,596,795 5,089,482). However, it is unknown if intranasal delivery of a composition containing multiple active agents, such as a GnRH compound and one or more hormonal agents, is suitable for contraception or for treatment of benign gynecological disorders, for contraception, or for hormone replacement therapy. For example, it is unknown if the presence of multiple agents in the formulation interfere with absorption of one or another of the agents. Formulations comprised of a GnRH compound and one or more hormonal agents that are sufficiently non-irritating to the nasal mucosa for commercial viability have also not been described. Nor have formulations comprised of an estrogenic compound and an androgenic compound such as testosterone that are therapeutically effective when delivered intranasally and that are sufficiently non-irritating to the nasal mucosa for commercial viability been described.

[0019] There remains a need in the art for a hormonal therapy for treatment of benign gynecological disorders that excludes a progestin, to avoid the conditions and symptoms, such as increased incidence of breast cancer, associated with this hormone. However, the prior art suggests that endometrial hyperplasia results from administration of unopposed estrogen. Thus, while the prior art recognizes the use of GnRH compounds for treatment of benign gynecological disorders, prior art compositions either included a progestin as an "add-back" compound or limited the treatment to women not at risk for endometrial stimulation, *i.e.*, women receiving an exogenously supplied progestin on a regular or periodic basis or women who have had a hysterectomy.

[0020] There is also a need for a need for a nasal preparation of GnRH compounds for treatment of benign gynecological disorders and for contraception. There is further a need for a nasal preparation of an estrogenic compound and an androgenic compound, and an optional progestin compound, in the form of a complex with a cyclodextrin.

Summary of the Invention

[0021] Accordingly, it is an object of the invention to provide a method of treating benign gynecological disorders by administering a composition comprised of a GnRH compound and an estrogenic compound and, optionally, an androgenic compound, in the absence of a co-administered or sequentially administered progestin.

[0022] It is another object of the invention to provide a method of preventing pregnancy, *i.e.,* a method of contraception, by administering a composition comprised of a GnRH compound and an estrogenic compound and, optionally, an androgenic compound, in the absence of a co-administered or sequentially administered progestin.

[0023] The present invention is based on the finding that women who are not receiving an exogenously supplied progestin can be treated with a GnRH compound combined with an estrogenic compound and, optionally, an androgenic compound for an extended period of time (i.e., 6 to 12 months or more) with no increase in the risk of endometrial hyperplasia. That is, treatment of a benign gynecological disorder with a composition comprised of a GnRH compound and an estrogenic compound and, optionally, an androgenic compound need not be accompanied by simultaneous administration of an exogenously supplied progestin in order to prevent endometrial hyperplasia. Similarly, administration of a GnRH compound and an estrogenic compound and, optionally, an androgenic compound for contraception need not be in conjunction with administration of an exogenously supplied progestin in order to prevent endometrial hyperplasia.

[0024] Accordingly, in one aspect, the invention includes an improvement in a method of treating benign gynecological disorders in a female. In the method, co-administration of a GnRH compound in an amount effective to suppress ovarian estrogen and progesterone production, and an estrogenic compound along with, optionally an androgen, in an amount effective to prevent signs and symptoms of estrogen deficiency and androgen deficiency, is extended to a female patient population of premenopausal women who are not receiving an exogenously supplied progestin on a regular or periodic basis and who do not have a history of endometrial hyperplasia, without a significant increase in the risk of endometrial hyperplasia relative to the patient population of women who are receiving an exogenously supplied progestin.

[0025] In one embodiment, the GnRH compound is an agonist or an antagonist. GnRH peptide agonist, and exemplary GnRH agonist compounds include deslorelin, leuprolide, nafarelin, goserelin, decapeptyl, buserelin, histrelin, gonadorelin, and analogs thereof. Exemplary GnRH antagonist compounds include azaline B, abarelix, cetrorelix,

degarelix, and analogs thereof. In a preferred embodiment, the GnRH compound is deslorelin, at a daily dose between 0.025 and 1.5 mg.

[0026] In another embodiment, the GnRH agonist is administered by an intranasal route. The co-administered estrogenic compound, in one embodiment, is also administered intranasally, and in another embodiment, it is administered transdermally. A preferred estrogenic compound is 17ß-estradiol. When administered transdermally, 17-β-estradiol is administered at a daily dose between about 0.025 mg and about 0.1 mg.

[0027] As noted above, the composition can optionally include an androgen. Thus, in one embodiment, the method includes co-administering an androgenic compound such as testosterone. The androgen can be administered intranasally or by a transdermal route with the estrogenic compound. When the androgen is testosterone it can be administered at a daily dose sufficient to increase the average serum testosterone level over 24 hours to the premenopausal range of about 15 ng/dL to 80 ng/dL.

[0028] In another embodiment, the GnRH agonist and the estrogenic compound are co-administered intranasally, in an aerosol spray containing a daily spray volume between about 30 and 200 µL, and between about 0.15 mg and 0.6 mg of 17ß-estradiol. In yet another embodiment, the intranasal administration further includes co-administering testosterone by an intranasal route, in an aerosol spray containing a daily spray volume between 30 and 200 µL, and between 0.15 mg and 0.6 mg of 17ß-estradiol and between 0.15 mg and about 1 mg of testosterone. In another embodiment, the androgen is present as a second or third steroid in a water-soluble complex with cyclodextrin.

[0029] In still another embodiment, the formulation further includes a progestin as a second or third steroid in the form of a water-soluble complex with the cyclodextrin.

[0030] The estrogenic compound and the second and or third steroid can have a combined molar occupancy with respect to the cyclodextrin that is greater than the molar occupancy achievable with any of the steroids alone.

[0031] It is another object of the invention to provide a bolus-form of delivery of a composition comprised of a GnRH compound and an estrogenic compound, and optionally an androgenic compound, that offers a therapeutic activity similar to that of a slow-release composition of the same active agents, with similar hormonal areas under the curve.

[0032] In another aspect, the nasal preparation described above when intranasally

administered as a daily bolus (i) is effective to achieve an average serum concentration over 24 hours of the estrogenic compound that is within 10% of the average serum concentration over 24 hours of the estrogenic compound when delivered transdermally and (ii) is as effective in preventing bone mineral density loss as transdermal administration of the estrogenic compound.

[0033] In another aspect, the invention includes a method of treating benign gynecological disorders in a patient population composed of premenopausal women who do not have a history of endometrial hyperplasia, and who are not receiving an exogenously supplied progestin on a regular or periodic basis. The method includes administering by daily intranasal administration, over an extended period of time between 6 and 12 months, a formulation containing a GnRH compound, in an amount effective to suppress ovarian estrogen and progesterone production, and an estrogenic compound and optionally an androgen, in an amount effective to prevent signs and symptoms of estrogen deficiency and androgen deficiency over a time period of between about 6 and 12 months.

[0034] In one embodiment, the nasal formulation is an aqueous formulation, and said administering is effective to deliver a daily spray volume between 30 and 200 μL.

[0035] In another embodiment, the spray volume administered includes between 0.15 and 0.6 mg of 17ß-estradiol. In yet another embodiment, the spray volume further includes testosterone in an amount between 0.15 mg and 1 mg.

[0036] The spray volume can further include, in another embodiment, a cyclodextrin, such as 2-hydroxypropyl-ß-cyclodextrin. Preferably, the 2-hydroxypropyl-ß-cyclodextrin, when present, is present in a mole ratio of cyclodextrin to total steroid of between 1:1 and 3:1. In one embodiment, the cyclodextrin is 2-hydroxypropyl-ß-cyclodextrin and is present in the formulation at a concentration between 50 and 300 mg/mL. In another embodiment, the 2-hydroxypropyl-ß-cyclodextrin has a degree of substitution between 2 and 8, more preferably between 5 and 8.

[0037] The nasal formulation can also take the form of an aerosolizable dry powder, as will be further described below. In one embodiment, the dry powder also includes testosterone, in a mole ratio of estrogenic compound: testosterone of between 1:1 and 1:2.

[0038] In another aspect, the invention includes an improvement in a method for contraception. The improvement comprises extending the contraceptive method of administering a GnRH compound and an estrogenic compound to women who are not receiving an exogenously supplied progestin on a regular or periodic basis. The method

includes administering by daily intranasal administration, over an extended period of time between about 6 and 12 months, a formulation containing a GnRH compound, in an amount effective to suppress ovarian estrogen and progesterone production, and an estrogenic compound, and optionally an androgen, in an amount effective to prevent signs and symptoms of estrogen deficiency and androgen deficiency over a time period of between about 6 and 12 months.

[0039] In one aspect, the invention includes a nasal spray formulation for use in female contraception or in the treatment of benign gynecological disorders, the composition comprising an aqueous medium having dissolved therein (i) a GnRH compound and (ii) an estrogenic compound present in the form of a water-soluble complex with a water-soluble cyclodextrin. The concentration of GnRH compound and estrogenic compound in the formulation are effective, when administered daily in the form of a liquid aerosol having a total liquid volume between 30 and 200 μL, and over an extended period of administration, to suppress ovarian estrogen and progesterone production and to prevent signs and symptoms of estrogen deficiency, without a significant increase in the risk of endometrial hyperplasia.

[0040] In another aspect, the invention includes an intranasal drug-delivery system for use in female contraception or in the treatment of benign gynecological disorders. The system is comprised of a nebulizer operable to deliver a selected volume between 30 and 200 μL of an aqueous formulation in the form of a liquid-droplet aerosol. Contained in the nebulizer is a liquid formulation composed of (i) a liquid carrier, (ii) a GnRH compound capable of suppressing ovarian estrogen and progesterone production, and (iii) an estrogenic compound capable of preventing signs and symptoms of estrogen deficiency when co-administered with the GnRH compound. The concentration of GnRH compound and estrogenic compound in the formulation are effective, when administered once daily in the form of a liquid aerosol having a total liquid volume between 30 and 200 μL, and over an extended period of administration, to suppress ovarian estrogen and progesterone production and to prevent signs and symptoms of estrogen deficiency, without a significant increase in the risk of endometrial hyperplasia.

[0041] Accordingly, it is an object of the invention to provide a nasal preparation having an estrogenic compound and an androgenic compound, and an optional progestin compound, in the form of a complex with a cyclodextrin.

[0042] It is another object of the invention to provide a bolus-form of delivery of a composition comprised of an estrogenic compound and an androgenic compound and, optionally a progestin compound, that offers a therapeutic activity similar to that of a

slow-release composition of the same active agents, with similar hormonal areas under the curves (AUCs) in a concentration-time plot of the two formulations or in achieving a similar biological effect such as amelioration of symptoms related to sex-steroid deprivation, specifically, loss of bone mineral density, atrophic vaginitis, and vasomotor instability.

[0043] In one aspect, the invention includes improvements in methods for contraception, for treatment of benign gynecological disorders, both in conjunction with a GnRH compound, and for hormone replacement for post-menopausal or surgically-postmenopausal women, where an estrogenic compound and an androgenic compound, such as testosterone, and an optional progestin compound, are administered, often on a long-term basis of longer than about 6 to about 12 months. The improvement comprises administering the estrogenic compound and the androgenic compound (an the optional progestin compound when present) intranasally in a once-daily bolus of an aqueous formulation containing the two or three compounds in the form of soluble complexes with a cyclodextrin. The amount of the two or three compounds administered is such as to produce estrogen and androgen and, optionally, progestin serum concentrations levels having substantially the same area-under-the-curve concentrations as are produced when therapeutically effective doses of the two or three compounds are administered transdermally. That is, administration of the estrogenic compound and the androgenic compound and, optionally, a progestin compound intranasally in bolus form achieves the same desired biological effect as that produced when the two or three compounds are administered transdermally.

[0044] The method of the invention, when used for treatment of a benign gynecological disorder or for contraception in conjunction with a GnRH compound, in one embodiment, includes administering the GnRH compound by any suitable route of administration, which may be different than an intranasal route employed for administration of the estrogenic compound and the androgenic compound and, optionally a progestin compound. The GnRH compound can be administered simultaneously or sequentially.

[0045] In one embodiment, the cyclodextrin is 2-hydroxypropyl-ß-cyclodextrin and is present at a concentration between about 50 mg/mL and 300 mg/mL. In another embodiment, 2-hydroxypropyl-ß-cyclodextrin has a degree of substitution of between 2 and 8, more preferably between 5 and 8.

[0046] In another embodiment, the estrogenic compound is 17ß-estradiol and the androgenic compound is testosterone and together they have a combined molar occupancy with respect to the cyclodextrin that is greater than the molar occupancy

achievable with either steroid alone. For example, the combined molar occupancy of the two steroids is greater than 50%, in one embodiment. In another embodiment, the combined molar occupancy of the two steroids is greater than 60%.

[0047] In another embodiment, the estrogenic compound is 17ß-estradiol at a daily dose between 0.15 mg and 0.6 mg and the androgenic compound is testosterone at a daily dose between 0.15 mg and 1 mg.

[0048] The mole ratio of 17ß-estradiol to testosterone is between 1:1 and 1:2, in some embodiments.

[0049] In yet other embodiments, the molar occupancies of 17ß-estradiol and testosterone are greater than 20% and 40%, respectively.

[0050] In another aspect, the invention includes an intranasal drug-delivery system for use in contraception or in treatment of benign gynecological disorders in conjunction with a GnRH compound, or in hormone replacement for postmenopausal or surgically postmenopausal women. The system is comprised of (a) a nasal-spray nebulizer effective to deliver a spray volume of between about 30 to about 200 µL, and (b) contained in the nebulizer, a drug formulation containing an estrogenic compound and an androgenic compound such as testosterone and, optionally, a progestin compound in an aqueous medium, in solubilized form complexed with a cyclodextrin. The amount of the two or three compounds administered in the spray volume is such as to produce estrogen and androgen, and progestin when present, serum concentrations having substantially the same area-under-the-curve concentrations as those produced when therapeutically effective doses of the two or three compounds are administered transdermally. That is, the biological effect achieved by intranasal administration of the two or three compounds is comparable to that produced when the two or three compounds are administered transdermally.

[0051] In one embodiment, the nasal preparation and the system are used on a long-term basis, *i.e.*, for longer than about 6 months, more preferably for longer than about 12 months.

[0052] In yet another aspect, the invention includes a method of formulating two or more different steroids in a water-soluble form suitable for uptake by a human subject through mucosal tissue. The method is comprised of forming an aqueous solution of a cyclodextrin and adding the first, second and, optionally, third steroid to the solution in amounts effective to achieve a combined molar occupancy of the two or three steroids which is greater than the molar occupancy achievable with any single steroid alone.

[0053] In one embodiment, the aqueous solution of cyclodextrin is heated to above

about 70°C prior to said adding, and the solution is cooled slowly after solubilization of the added steroids.

[0054] In another embodiment, the first steroid is added to the solution until a maximum or near-maximum molar occupancy is reached, then the second and, optionally a third steroid, is/are added until a combined maximum or near-maximum molar occupancy is reached.

[0055] The cyclodextrin can be 2-hydroxypropyl-ß-cyclodextrin, the estrogenic compound can be 17ß-estradiol, the second steroid can be testosterone, and the third steroid can be progesterone.

[0056] These and other objects and features of the invention will be more fully appreciated when the following detailed description of the invention is read in conjunction with the accompanying drawings and examples.

Brief Description of the Drawings

[0057] Figures 1A and 1B are bar graphs showing the cumulative area under the curve from 0 to 360 minutes in pg·min/mL of serum estradiol (Fig. 1A) and in ng·min/dL of serum testosterone (Fig. 1B) for six patients treated with an intranasal preparation comprised of the GnRH compound deslorelin, estradiol, and testosterone. The preparation was administered twice (days 1 and 8), one week apart.

[0058] Fig. 2 is a bar graph showing the molar ratio of steroid to 2-hydroxypropyl-β-cyclodextrin for testosterone (T/HPβCD), for estradiol (E2/HPβCD), and for testosterone and estradiol in combination where the two steroids are added to the 2-hydroxypropyl-β-cyclodextrin solution simultaneously (T+E2/HPβCD) or added sequentially (E2 then T/HPβCD).

Detailed Description of the Invention

I. Definitions

[0059] The phrase "regular basis" intends an on-going, and predictably scheduled action. A "regular basis" can be periodic when the action is not necessarily continuous, but the action occurs at a period that is predictable or scheduled in an on-going fashion.

[0060] A "periodic basis" intends an action that is intermittent and predictably scheduled.

[0061] An "extended time period" intends a period of more than about 4 months, preferably more than about 6 months.

[0062] The term "premenopausal" refers to the period corresponding to a woman's

reproductive years defined as the time between onset of menses (menarche) and the final episode of menstrual bleeding (menopause).

[0063] The phrase "amount effective to prevent signs and symptoms of estrogen deficiency" refers to a dose of a therapeutic compound that inhibits or minimizes clinically-recognized markers of estrogen deficiency, including but not limited to symptoms typically associated with menopause, such as vasomotor instability, bone loss, and/or urogenital atrophy.

[0064] The phrase "amount effective to prevent signs and symptoms of androgen deficiency" intends a dose of a therapeutic compound that inhibits or minimizes clinically-recognized indicators of androgen deficiency, and in particular the clinical indicators of testosterone deficiency such as bone loss and decreased libido. Such signs and symptoms typically overlap with or are a subset of the signs and symptoms of estrogen deficiency.

[0065] The phrase "amount effective to suppress ovarian estrogen and progesterone production" refers to a dose of a therapeutic compound, and in particular to a dose of a GnRH compound, that reduces serum estrogen levels such that one or more symptoms typically associated with menopause, such as vasomotor instability, bone loss, decreased libido, vaginal dryness, and/or urogenital atrophy, are observed. Typically, a sustained serum estrogen level of less than about 30 pg/mL, more typically of less than about 20 pg/mL, is considered as evidence that ovarian estrogen production is suppressed.

[0066] The phrase "amount effective to suppress ovarian progesterone production" refers to a dose of a therapeutic compound, and in particular to a dose of a GnRH compound, that maintains serum progesterone at a level consistent with anovulation. Typically, a sustained serum progesterone level of less than about 80 ng/dL, more typically of less than about 50 ng/dL, is considered as evidence that ovarian progesterone production is suppressed.

[0067] The terms "progestin" and "progestogen" are used interchangeably.

[0068] The term "GnRH compound" as used herein intends peptide and non-peptide GnRH analogs, and includes agonists and antagonists. Exemplary non-peptide analogs are described, for example, in U.S. Patent No. 6,346,534. Peptide analogs are widely reported in the literature and examples are provided herein.

[0069] The phrase "without significant risk of endometrial hyperplasia" intends a risk of developing simple endometrial hyperplasia that is less than the average risk of a given population of women. For example, the average risk of hyperplasia after one year of

treatment with unopposed estrogen in the general population of postmenopausal (naturally or surgically menopausal) women treated with unopposed estrogen (0.625 mg dose) is about 30% (Gefland, M. *et al. Obstetrics & Gynecology*, 74:398 (1989)). Thus, 'no significant risk of endometrial hyperplasia', with respect to postmenopausal women treated with 0.625 mg daily unopposed estrogen, would be less than 30%, more preferably less than 20%, still more preferably less than 10%, even still more preferably less than 5%, of a test population developing simple endometrial hyperplasia.

II. Method of Treatment

[0070] As noted above, in one aspect the invention includes a method of treating a benign gynecological disorder in a woman by administration of a GnRH compound in combination with an estrogenic compound and optionally an androgenic compound. In this section, each component in the composition and exemplary routes of administration will be described.

[0071] The invention further includes a steroid nasal preparation for use in conjunction with a GnRH compound, comprising an estrogenic compound and an androgenic compound, and an optional progestin compound. For example, the steroid nasal preparation finds use as add-back hormone replacement in women who have hormone production suppressed with a gonadotropin releasing hormone (GnRH) compound. "GnRH compound" refers to peptide and non-peptide GnRH analogs, including agonists and antagonists. These compounds are administered, for example, for female contraception and in the treatment of benign gynecological disorders. Thus, women currently taking a GnRH compound for treatment of a benign gynecological disorder or for contraception are candidates for treatment with the steroid nasal preparation described herein. The steroid nasal preparation also is suitable for use in hormone replacement therapy for both postmenopausal and surgically postmenopausal women. The steroid nasal preparation is also suitable in peri-menopausal women, *i.e.*, women entering menopause who have a low hormone level, and in women with a low hormone level as a result of another condition, disorder, or treatment regimen.

A1. Composition Components: GnRH Compound

[0072] The composition for use in one method of the invention comprises a GnRH compound. Native GnRH is a decapeptide comprised of naturally-occurring amino acids having the L-configuration, except for the achiral amino acid glycine. The sequence of GnRH is (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (SEQ ID NO:1). A large

number of analogs of this natural peptide have been prepared and are effective to inhibit the release and/or the action of GnRH. Analogs having agonist and antagonist activity have been described, and as used herein, the term "a GnRH compound" or "GnRH compounds" intends agonists and antagonists, synthetically prepared or naturally-occurring, peptides and non-peptide compounds alike. The following description focuses in particular on GnRH agonists, however, it will be appreciated that native GnRH, GnRH antagonists, such as azaline B, abarelix, cetrorelix, and degarelix, and other GnRH analogs are also suitable for use in the composition and method of treatment. Further, the following discussion focuses on peptide analogs, however, it will be appreciated that non-peptide compounds, such as those disclosed in U.S. Patent No. 6,346,534, are also contemplated.

[0073] GnRH agonists are compounds that work in two phases. The first phase stimulates the ovaries to produce more estradiol. During the second phase, the messenger hormones that control the ovaries are no longer produced, resulting in a drop in estrogen. An exemplary agonist is obtained by changing the 6-position residue in the naturally-occurring GnRH from Gly to a D-amino acid, to give a GnRH analog having a sequence (pyro)Glu-His-Trp-Ser-Tyr-X-Leu-Arg-Pro-Gly-NH_2 (SEQ ID NO:2), where X represents an amino acid in the D-configuration. When X is D-Leu the analog is known as Lupron™ and is commercially available from TAP Pharmaceuticals (Lake Forest, IL). Agonists often have the N-terminus prolyl modified with an n-ethylamide addition. For example, the agonist deslorelin is (pyro)Pro-His-Trp-Ser-Tyr-DTrp-Leu-Arg-Pro-ethylamide (SEQ ID NO:3). Another exemplary analog is where the 6-position residue is D-Ala to give a peptide having the following sequence: (pyro)Glu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-Gly-NH_2 (SEQ ID NO:4; U.S. Pat. No. 4,072,668). Another exemplary agonist is obtained by eliminating the Gly-NH_2 in position 10 to give a nonapeptide as an alkyl, cycloalkyl, or fluoroalkylamide, or by replacing Gly-NH_2 by an α-azaglycine amide. Modifications to the naturally-occurring GnRH sequence at positions 1 and 2 are also possible. A number of GnRH agonists are described in the art, many of which are commercially available, and include deslorelin, leuprolide, nafarelin, goserelin, decapeptyl, buserelin, histrelin, and gonadorelin, and analogs thereof.

[0074] The amount of GnRH compound effective to achieve the desired suppression of ovarian estrogen production may readily be determined with respect to any given GnRH compound and for any given mammal. The dose range depends upon the particular GnRH compound used and may also depend upon patient characteristics, such as age and weight. Further, the effective amount of GnRH compound also

depends upon route of administration. Determination of an effective dose range after consideration of these factors is routine for those of skill in the art.

[0075] In one embodiment, the dose of the GnRH compound is preferably sufficient to suppress ovarian estrogen and progesterone production, so that estrogen effects are predictably related to the co-administered estrogenic compound, described below. The amount of GnRH compound effective to achieve the desired suppression of ovarian estrogen production may readily be determined with respect to any given GnRH compound and for any given mammal. The dose range depends upon the particular GnRH compound used and may also depend upon patient characteristics, such as age and weight. Further, the effective amount of GnRH compound also depends upon route of administration. Determination of an effective dose range after consideration of these factors is routine for those of skill in the art.

[0076] By way of example of a specific formulation, the amount of GnRH compound when the GnRH compound is deslorelin in a daily nasal spray formulation with a volume between about 30 to 200 μL can deliver a daily dose of GnRH compound of between about 0.025 mg to about 1.5 mg, more preferably from about 0.025 mg to about 0.1 mg. It will be appreciated that the daily spray volume can be administered in one, two, or more separate deliveries to achieve the desired total daily spray volume. It will further be appreciated that the spray volume and the amount of GnRH compound in the nasal formulation are both individually adjustable to achieve the desired daily dosage.

A2. Composition Components: Estrogenic Compound

[0077] The composition for use in the methods of the invention includes an effective amount of an estrogenic compound. The estrogenic compound is effective to prevent symptoms and signs of estrogen deficiency including bone loss, vaginal atrophy, and hot flashes. The estrogenic compound may be present in a steroid composition.

[0078] The estrogenic compound can be a single-component natural or synthetic estrogen composition, or a combination of such compounds. As used herein, the term "estrogenic compound" refers to both natural and synthetic materials having activity to mitigate the signs and symptoms of estrogen deficiency. Natural and synthetic estrogenic compositions which can be used according to the invention described herein include natural estrogenic hormones and congeners, including but not limited to estradiol, estradiol benzoate, estradiol cypionate, estradiol valerate, estrone, diethylstilbestrol, piperazine estrone sulfate, ethinyl estradiol, mestranol, polyestradiol phosphate, estriol, estriol hemisuccinate, quinestrol, estropipate, pinestrol, and estrone

potassium sulfate. Equine estrogens, such as equilelinin, equilelinin sulfate, and estetrol, and synthetic steroids combining estrogenic, androgenic, and progestogenic properties such as tibolone may also be employed.

[0079] Typical dose ranges for estrogenic compounds depend on the compound and on the characteristics of the patient. For an adult human female patient treated with a transdermal 17ß-estradiol preparation, a typical dose range is one that maintains a serum level of estradiol of about 25 to about 140 pg/mL, more preferably between about 30 to about 50 pg/mL. A specific example of a composition containing an estrogenic compound is one comprised of a GnRH agonist and 17-β-estradiol. The two compounds, along with other optional excipients or an androgenic compound, are formulated for transdermal or intranasal delivery. In a transdermal formulation, a preferred daily dosage range for 17-β-estradiol is between about 0.025 mg and 0.1 mg. For an intranasal preparation, a preferred daily dosage range for 17-β-estradiol is between about 0.15 mg and 0.6 mg.

[0080] As discussed below, in one embodiment, the estrogenic compound is preferably co-administered from the same delivery vehicle or via the same route as the GnRH compound. However, delivery of the estrogenic compound can be from a different vehicle and/or by a different route than the GnRH compound, and some examples of such "mixed modes" of administration are provided below. As can be appreciated, the composition comprised of a GnRH compound and an estrogenic compound suppresses gonadotropin activity while providing a replacement of estrogen to minimize or eliminate the side effects associated with suppression of gonadotropin activity.

A3. Composition Components: Androgenic Compound

[0081] The composition comprised of a GnRH compound and an estrogenic compound can optionally include an androgenic compound. Additionally, the steroid composition may further include an androgenic compound. When present in the composition, the androgenic compound is in an amount effective to increase a patient's androgen level to a level not exceeding a "normal" premenopausal level, and in particular in concert with the estrogenic composition to maintain bone mineral density. Such "normal" androgen levels are on the order of about 15 ng/dL to about 80 ng/dL for testosterone.

[0082] Suitable androgenic compounds for use in the composition include but are not limited to testosterone, androstenedione, dihydrotestosterone, testosterone

propionate, testosterone enanthate, testosterone cypionate, methyltestosterone, danazol, dromostanolone propionate, ethylestrenol, methandriol, nandrolone decanoate, nandrolone phenpropionate, oxandrolone, oxymethalone, and stanozolol.

[0083] Typical dose ranges for androgenic hormones depend upon the choice of compound and the individual patient. For an adult human female administered testosterone, typical doses are administered to provide average serum levels of testosterone of from about 15 ng/dL to about 80 ng/dL, and preferably about 40 ng/dL to about 60 ng/dL. A specific example of a composition containing an androgenic compound is one comprised of a GnRH agonist and 17-β-estradiol and testosterone. The compounds, along with other optional excipients, are formulated for delivery transdermally or intranasally. For an intranasal preparation, a typical daily dose of testosterone can range from about 0.15 mg to about 1 mg. In one embodiment, the compounds, along with other optional excipients, are formulated for delivery intranasally, and exemplary formulations are described below.

A4. Composition Components: Progestin Compound

[0084] The composition comprised of a GnRH compound and an estrogenic compound, in some embodiments, can further include a progestin. The steroid composition comprised of an estrogenic compound and an androgen can optionally include a progestin. Formulations that include a progestin can be administered for a limited period of time, for example on the order of 5 to 20 days, and preferably 10 to 15 days after each extended treatment regimen of, for example, about 4 months, more preferably greater than about 6 months, and more specifically, of from about 4 months to about 12 months. The progestin is provided in an amount effective to minimize or eliminate the occurrence of endometrial hyperplasia by substantially reducing the possibility of endometrial hyperstimulation which may occur during prolonged treatment with estrogenic steroids without a progestin.

[0085] Suitable progestational agents (progestins) include but are not limited to dydrogesterone, ethynodiol diacetate, hydroxyprogesterone caproate, medroxyprogesterone acetate, norethindrone, norethindrone acetate, norethynodrel, norgestrel, progesterone, and megestrol acetate. Typical dose ranges for progestins depend upon the choice of steroid and the individual patient. Doses are selected as adequate to produce a secretory uterine endothelium after the time interval of progestogen treatment (about 5 to about 20 contiguous days, and preferably about 10 to about 15 contiguous days). The serum level of progesterone is generally less than about 50 ng/dL after the time interval of progestin treatment.

B. Exemplary Modes of Administration

[0086] As noted above, the compositions described herein are beneficial for use in female contraception and/or in the treatment of benign gynecological disorders. The compositions described herein are further beneficial for hormone replacement therapy. In general, the compositions can be administered by any vehicle or route that achieves efficacious therapy. Parenteral (*e.g.*, non-gastrointestinal, such as subcutaneous, intramuscular, intravenous), transdermal, pulmonary, mucosal (nasal, vaginal, rectal, buccal) routes of delivery are contemplated and preparation of suitable dosage forms can be prepared by methods well-known in the pharmaceutical art, for example, as described in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Co., Easton, Pa. (1985). Some exemplary formulations for various routes of administration are discussed below.

[0087] For example, in one embodiment it is contemplated that the composition is administered mucosally by contacting the composition in a suitable dosage form with mucosal tissue of the vagina, nose, rectum, or mouth. In a preferred embodiment, the composition is administered via the nasal mucosa, *e.g.*, intranasally. The nasal mucosa provides a useful anatomical site for systemic delivery. The nasal tissue is highly vascularized, providing an attractive site for rapid and efficient absorption. The adult nasal cavity has a capacity of around 20 mL, with a large surface area of approximately 180 cm^2 for drug absorption, due in part to the microvilli present along the psuedostratified columnar epithelial cells of the nasal mucosa.

[0088] A nasal preparation comprised of the compositions described above can take a variety of forms for administration in nasal drops, nasal spray, gel, ointment, cream, powder or suspension, using a dispenser or other device as needed. A variety of dispensers and delivery vehicles are known in the art, including single-dose ampoules, atomizers, nebulizers, pumps, nasal pads, nasal sponges, nasal capsules, and the like.

[0089] More generally, the preparations can take a solid, semi-solid, or liquid form. In the case of a solid form, the components may be mixed together by blending, tumble mixing, freeze-drying, solvent evaporation, co-grinding, spray-drying, and other techniques known in the art. Such solid state preparations preferably provide a dry, powdery composition with particles in the range of between about 20 to about 500 microns, more preferably from 50 to 250 microns, for administration intranasally.

[0090] A semi-solid preparation suitable for intranasal administration can take the form of an aqueous or oil-based gel or ointment. For example, the components described above can be mixed with microspheres of starch, gelatin, collagen, dextran,

polylactide, polyglycolide, or other similar materials that are capable of forming hydrophilic gels. The microspheres can be loaded with drug, and upon administration form a gel that adheres to the nasal mucosa.

[0091] In a preferred embodiment, the nasal preparation is in liquid form, which can include an aqueous solution, an aqueous suspension, an oil solution, an oil suspension, or an emulsion, depending on the physicochemical properties of the composition components. The liquid preparation is administered as a nasal spray or as nasal drops, using devices known in the art, including nebulizers capable of delivering selected volumes of formulations as liquid-droplet aerosols. For example, a commercially available spray pump with a delivery volume of 50 μL or 100 μL is available from, for example, Valois (Congers, NY) with spray tips in adult size and pediatric size. In one embodiment, the composition comprised of a GnRH agonist and an estrogenic compound are co-administered intranasally via an aerosol spray in a daily volume of between about 10 to 500 μL, more preferably between about 30 to about 200 μL.

[0092] The liquid preparation can be produced by known procedures. For example, an aqueous preparation for nasal administration can be produced by dissolving, suspending, or emulsifying the polypeptide and the steroid compounds in water, buffer, or other aqueous medium, or in a oleaginous base, such as a pharmaceutically-acceptable oil like olive oil, lanoline, silicone oil, glycerine fatty acids, and the like.

[0093] It will be appreciated that excipients necessary for formulation, stability, and/or bioavailability can be included in the preparation. Exemplary excipients include sugars (glucose, sorbitol, mannitol, sucrose), uptake enhancers (chitosan), thickening agents and stability enhancers (celluloses, polyvinyl pyrrolidone, starch, etc.), buffers, preservatives, and/or acids and bases to adjust the pH.

[0094] In a preferred embodiment, an absorption promoting component is included. Exemplary absorption promoting components include surfactant acids, such as cholic acid, glycocholic acid, taurocholic acid, and other cholic acid derivatives, chitosan, and cyclodextrins. In a preferred embodiment, a cyclodextrin is included in the preparation. Cyclodextrins are cyclic oligosaccharides of α-D-gluco-pyranose and can be formed by the catalytic cycilization of starch. Due to a lack of free rotation about the bonds connecting the glycopyranose units, cyclodextrins are toroidal or cone shaped, rather than cylindrical. The cyclodextrins have a relatively hydrophobic central cavity and a hydrophilic outer surface. The hydrophobic cage-like structure of cyclodextrins has the ability to entrap a variety of guest compounds to form host-guest complexes in the solid state and in solution. These complexes are often termed inclusion complexes and the

guest compounds are released from the inclusion site.

[0095] The most common cyclodextrins are α-, β-, and γ-cyclodextrin, which consist of six, seven, or eight glucopyranose units, respectively. Cyclodextrins containing nine, ten, eleven, twelve, and thirteen glucopyranose units are designated δ-, ε-, ξ-, η-, and θ-cyclodextrin, respectively. Characteristics of α-, β-, γ-, and δ-cyclodextrin are shown in Table 1.

Table 1: Cyclodextrin Characteristics

	α- cyclodextrin	β-cyclodextrin	γ-cyclodextrin	δ-cyclodextrin
no. of glucopyranose units	6	7	8	9
molecular weight (Daltons)	972	1135	1297	1459
central cavity diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3	10.3-11.2
water solubility (at 25°C, g/100 mL)	14.5	1.85	23.2	8.19

[0096] Derivatives formed by reaction with the hydroxyl groups lining the upper and lower ridges of the toroid are readily prepared and offer a means of modifying the physicochemical properties of the parent cyclodextrins. The parent cyclodextrins, and in particular β-cyclodextrin, have limited aqueous solubility. Substitution of the hydroxyl groups, even with hydrophobic moieties such as methoxy and ethoxy moieties, typically increases solubility. Since each cyclodextrin hydroxyl group differs in chemical reactivity, reaction with a modifying moiety usually produces an amorphous mixture of positional and optical isomers. The aggregate substitution that occurs is described by a term called the degree of substitution. For example, a 2-hydroxypropyl-β-cyclodextrin with a degree of substitution of five would be composed of a distribution of isomers of 2-hydroxypropyl-β-cyclodextrin in which the average number of hydroxypropyl groups per 2-hydroxypropyl-β-cyclodextrin molecule is five. Degree of substitution can be determined by mass spectrometry or nuclear magnetic resonance spectroscopy. These methods do not give information as to the exact location of the substituents (C1, C2, C3, etc.) or the distribution of the substituents on the cyclodextrin molecule (mono, di, tri, poly). Theoretically, the maximum degree of substitution is 18 for α-cyclodextrin, 21 for β-cyclodextrin, and 24 for γ-cyclodextrin, however, substituents with hydroxyl groups present the possibility for additional hydroxylalkylations.

[0097] The cyclodextrin used in the present invention is preferably an α-, β-, or γ-cyclodextrin. The cyclodextrin is selected for use depending on which cyclodextrin binds the guest compounds and yields the desired bioavailability. In a preferred embodiment,

a derivative of a cyclodextrin is selected, and derivatives such as hydroxypropyl, dimethyl, and trimethyl substituted cyclodextrins are contemplated, as are cyclodextrins linked with sugar molecules, sulfonated cyclodextrins, carboxylated cyclodextrins, and amino derivatives such as diethylamino cyclodextrins. In a preferred embodiment, the cyclodextrin is a β-cyclodextrin, and in a more preferred embodiment, the cyclodextrin is 2-hydroxypropyl-β-cyclodextrin. In yet another embodiment, the 2-hydroxypropyl-β-cyclodextrin has a degree of substitution between 2 and 8, more preferably between 4 and 8, most preferably between 5 and 8.

[0098] In a study performed in support of the invention, an intranasal formulation comprised of the GnRH compound deslorelin and of estradiol, testosterone, and cyclodextrin was prepared, as described in Example 1 and further discussed below.

[0099] In another study performed in support of the invention, the solubility of steroids estradiol and testosterone, alone and in combination, in varying concentrations of 2-hydroxypropyl-β-cyclodextrin was determined. As described in Example 5, defined amounts of each steroid were added to 1 mL of 2-hydroxypropyl-β-cyclodextrin in water. The solubility of the steroids was determined, and the results are shown in Tables 2A and 2B.

[00100] Table 2A shows the solubility of 17-ß-estradiol and testosterone individually in aqueous solutions of 2-hydroxypropyl-β-cyclodextrin. The last two columns in the Table 2A show the molar ratio of each steroid to the cyclodextrin. The molar occupancy of 17-ß-estradiol with respect to 2-hydroxypropyl-β-cyclodextrin, averages approximately 0.21. The molar occupancy of testosterone with respect to 2-hydroxypropyl-β-cyclodextrin averages approximately 0.39.

Table 2A: Solubility of 17-ß-Estradiol and Testosterone in 2-Hydroxypropyl-ß-cyclodextrin

HPβCD* mg/mL	17-ß-Estradiol solubility mg/mL	Testosterone solubility mg/mL	Molar Ratio Estradiol/HPβCD	Testosterone/HPβCD
10	0.414	0.810	0.21	0.39
40	1.605	2.541	0.20	0.30
70	2.763	5.626	0.20	0.38
100	4.062	6.819	0.21	0.33
130	5.494	11.649	0.21	0.43
160	6.841	13.866	0.22	0.41
190	8.379	16.522	0.22	0.42
220	9.330	18.604	0.21	0.40
250	11.031	21.684	0.22	0.41

*2-hydroxypropyl-β-cyclodextrin

[00101] Table 2B shows the solubility of 17-ß-estradiol as a first steroid and testosterone as a second steroid in aqueous 2-hydroxypropyl-β-cyclodextrin. The last three columns show the molar ratios of each steroid individually in the 2-hydroxypropyl-β-cyclodextrin solution and of the two steroids together in the solution. The data shows that the combined molar occupancy of the two steroids together, average approximately 0.48, is greater than the molar occupancy achieved with either steroid alone (Table 2A).

Table 2B: Molar Occupancy of 17-ß-Estradiol and Testosterone in 2-Hydroxypropyl-ß-cyclodextrin

HPβCD * mg/mL	Estradiol and Testosterone solubility mg/mL		Molar Ratio		
	Estradiol	Testosterone	Estradiol/ HPβCD	Testosterone/ HPβCD	Estradiol & Testosterone/ HPβCD
10	0.164	0.658	0.08	0.31	0.40
40	0.834	2.819	0.11	0.34	0.44
70	1.562	5.073	0.11	0.35	0.46
100	2.157	7.113	0.11	0.34	0.45
130	3.202	10.552	0.12	0.39	0.51
160	4.053	13.422	0.13	0.40	0.53
190	4.796	15.742	0.13	0.40	0.52
250	5.774	19.986	0.12	0.38	0.50

*2-hydroxy-propyl-β-cyclodextrin

[00102] The molar ratio data of Tables 2A and 2B are presented graphically in Fig. 2. The figure also shows the molar ratio determined in another study where estradiol was first added to the aqueous 2-hydroxypropyl-β-cyclodextrin solution, followed by addition of testosterone. The molar occupancy of the two steroids in combination is similar, regardless of the sequence of addition of the steroids to the 2-hydroxypropyl-β-cyclodextrin solution.

[00103] In another study, the solubility of 17-ß-estradiol and testosterone, alone and in combination, as a function of degree of substitution of 2-hydroxypropyl-β-cyclodextrin was evaluated. Solutions of 2-hydroxypropyl-β-cyclodextrin with degrees of substitution of 5.5, 6.1, and 6.8 were prepared and the maximum concentration of estradiol and testosterone that could be solubilized was determined. There was a slight trend for the 2-hydroxypropyl-β-cyclodextrin with a lower degree of substitution to solubilize moresteroid, however, the trend was not statistically significant.

[00104] Another exemplary mode of administration suitable for the method of the invention is an intravaginal device, such as an intravaginal ring or a vaginal thin-film

laminate. Vaginal rings are well-known in the art (see for example Duncan *et al.*, *Silicone Based Release Systems,* in POLYMERS IN MEDICINE AND SURGERY, Kronenthal *et al.* Eds., Plenum, NY, 1975, p. 205; U.S. Patent Nos. 4,012,496; 5,869,081) and are made by polymerizing in a simple mold a mixture of the drug dispersion and a suitable polymer, such as silicone rubber. The ring or device is inserted into the vaginal cavity and retained there for a desired period of time for administration of the compounds incorporated into the device. Vaginal devices are described in LONG-ACTING CONTRACEPTIVE DELIVERY SYSTEMS, Zatuchni, G., L., *et al.* Eds., 1984, and the particular chapters by Jackanicz, T.M., "Vaginal Ring Steroid-Releasing Systems, p. 201-212; by Diczfalusy & Landgren, "Some Pharmacokinetic and Pharmacodynamic Properties of Vaginal Delivery Systems that Release Small Amounts of Progestogens at a Near Zero-Order Rate", p. 213-227; and by Roy & Mishell, "Vaginal Ring Clinical Studies: Update", p. 581-594, are incorporated by reference herein.

[00105] Another exemplary mode of administration is transdermal. Transdermal administration is one approach to achieving a constant blood level of drug in a patient for a period of time. Transdermal administration offers, in addition to the benefit of a more constant blood level, other benefits such as a more efficient utilization of the drug, the potential for localized, site specific delivery, and less frequent administration (Baker, R. W., CONTROLLED RELEASE OF BIOLOGICALLY ACTIVE AGENTS, John Wiley and Sons, New York, (1987) p. 5-10). More efficient utilization of the drug is an important benefit, since often less drug, when administered in a controlled release manner, is required to produce a given duration of effect than when administered by another route. This is particularly true if the half-life of the drug is short compared with the desired treatment period. Since the drug is utilized more efficiently, a considerably lower dose may be required, depending on the drug half-life and the desired time of treatment.

[00106] Transdermal delivery devices, also referred to as transdermal patches, have been widely described (see, for example, Baker, R.W., *Id.*). The transdermal device can be a simple adhesive matrix type device with the active agents incorporated into the adhesive layer, or a more complicated device with one or more drug reservoirs defined by an impermeable backing layer and a retaining membrane. Design of the device and selection of suitable materials is readily accomplished by those of skill in the art. An exemplary device for use in the present invention is one designed to administer to a patient a dose of GnRH and an estrogenic compound and, optionally, an androgenic compound in an amount sufficient to treat a benign gynecological disorder and/or achieve contraception.

[00107] The present invention contemplates administration of the GnRH compound and

the estrogenic compound, optionally containing an androgen, as a single composition by a single route of administration. For example, a dry powder comprised of the GnRH compound, the estrogenic compound, and, optionally, an androgen, and any desired excipients or enhancers, such as cyclodextrin, are mixed into a dosage form suitable for co-administration intranasally in the form of a dry aerosol. Or, for example, the same compounds are formulated into a liquid preparation for intranasal administration as a spray. Or, for example, the same compounds are incorporated into a transdermal device for co-administration of the GnRH, estrogenic compound, and the androgenic compound, if present, via the skin.

[00108] The invention further contemplates administration of the GnRH compound and the estrogenic compound, optionally containing an androgenic compound, in the form of two or more compositions for administration by two or more routes of administration. For example, the GnRH compound can be formulated into an intranasalpreparation, and the estrogenic compound and, optionally, the androgenic compound can be formulated into a transdermal device. The two formulations are administered to a patient for treatment of a benign gynecological disorder or for contraception. That is, the GnRH is administered intranasally as needed to achieve the desired daily dose, and the estrogenic compound and, optionally, the androgenic compound is administered transdermally. It will be appreciated that the androgenic compound can be included in either formulation, preferably in the transdermal device, or administered as a separate, third composition by any suitable route. Other 'mixed modes' of administration will be readily apparent to those of skill in the art.

C. *In vivo* Treatment Method

[00109] As noted above, the invention provides for an improvement in a method of treating benign gynecological disorders and for an improvement in a method of contraception, where a GnRH compound and an estrogenic compound and, optionally, an androgenic compound are administered to women in conjunction with an exogenously supplied progestin on a regular or periodic basis. The improvement consists of administering the GnRH compound and the estrogenic compound and, optionally, an androgenic compound, to women who are not receiving an exogenously supplied progestin on a regular or periodic basis. As will be described below, the improved method of treatment finds basis in the discovery that an exogenously supplied progestin is not required on a regular or periodic basis to prevent endometrial hyperplasia.

[00110] Thus, in a preferred embodiment, the improved method described herein extends treatment of benign gynecological disorders by administration of a GnRH compound and an estrogenic compound and, optionally, an androgenic compound to women not receiving an exogenously supplied progestin on a regular or periodic basis. However, it is to be understood that women treated in accord with the invention may be treated with an exogenously supplied progestin on an intermittent basis. That is, a physician may prescribe an occasional progestin to induce shedding of the endometrium or to preserve efficacy of the GnRH-estrogenic (and optionally androgenic) composition. Typically, the GnRH compound and the estrogenic compound and, optional androgenic compound are administered for an extended period of time, *e.g.*, longer than about 6 months to 12 months, and, if, for example, shedding of the endometrium is desired, a progestin can be taken for a defined interval of time. Thus, the invention further contemplates treatment of a benign gynecological disorder or contraception by administration of the GnRH compound and the estrogenic compound and optional androgenic compound, where a progestin is prescribed on an intermittent, *i.e.*, irregular, non-predictable, basis.

[00111] In another embodiment, the invention provides a method for formulating two or more steroids in a water-soluble form suitable for uptake through mucosal tissue of a subject. The selected steroids are added to an aqueous solution of a cyclodextrin simultaneously or sequentially, to achieve a molar occupancy of the steroids that is greater than the molar occupancy of any one of the steroids alone. The invention contemplates selection of an estrogenic compound, an androgenic compound, and/or a progestin compound as suitable steroids. When the steroids are added sequentially, the first steroid is added in an amount sufficient to reach a maximum or near-maximum molar occupancy of the steroid in the cyclodextrin. Then the second steroid is added in an amount sufficient to reach the combined maximum or near-maximum molar occupancy of the steroids in the cyclodextrin. In preparing the formulation, the solution of cyclodextrin can be heated prior to adding the steroids, and heating to above about 70°C is usually suitable to enhance solubilization if needed. After addition of the two or more steroids the solution can also be slowly cooled.

[00112] In studies performed in support of the invention, the GnRH compound deslorelin was administered intranasally to patients, both in the presence and absence of estradiol and/or testosterone. These studies will now be described.

[00113] Intranasal administration of deslorelin alone is described in Example 2. The objective of this study was to determine an appropriate dose of the GnRH compound

deslorelin effective to control the heavy bleeding secondary to uterine leiomyomata (fibroids). As described in Example 2, female patients were treated with deslorelin administered daily via intranasal delivery, at a dose of 0.5 mg, 1.0 mg, or 2.0 mg. The compound was administered using a commercially available nasal sprayer that delivered a 50 μL spray volume. The daily dose was administered by application of 50 μL to each nostril once per day, for a total daily spray volume of 100 μL. During the 12 week treatment period the patients kept daily bleeding calendars and underwent clinical assessments at scheduled intervals. Clinical assessments included grading of nasal irritation (Table 5A), determination of uterus size (Table 5B), and serum hormonal levels (Table 5C). These clinical data are presented in the indicated tables in Example 2, along with the bleeding scores (Table 5D).

[00114] With respect to nasal irritation, the data (Table 5A in Example 2) indicates that subjects experienced none or slight irritation at deslorelin dosages of 0.5 mg and 1 mg. Some of the subjects treated with a deslorelin dose of 2 mg experienced more frequent irritation.

[00115] Table 5B in Example 2 also shows a reduction in uterine volume (calculated from ultrasound determined dimensions), with the reduction directly correlating to deslorelin dose.

[00116] Serum levels of estradiol, progesterone, and testosterone are shown in Table 5C in Example 2. Reduction in estradiol levels was progressive and dose dependent over the 12 week period. Progesterone levels followed a similar pattern with progressive suppression with higher dose and longer time. Testosterone levels are also similar.

[00117] With respect to bleeding scores (Table 5D in Example 2), the patients treated with the GnRH compound had a marked improvement in their bleeding score at the end of the three month study.

[00118] In summary, all tested doses were partially or completely effective, as evidenced by a reduction of bleeding, and uterine size. This effect correlates with a reduction of estrogen and progesterone. Based on the bleeding scores, a 1.0 mg dose appears to offer a slight advantage compared with the 0.5 mg dose. Uterine size and rates of change of estrogen and testosterone levels are clearly dose related over the range studied.

[00119] Another study performed in support of the invention is described in Example 3. In this study, the GnRH compound deslorelin, estradiol, and testosterone were co-administered as an "all-in-one" nasal spray to oophorectomized women. Each woman was treated with 50 μL of a nasal spray preparation, prepared as described in Example 1,

delivered as a single dose on two occasions separated by one week. The 50 μL dose delivered 1 mg deslorelin, 50 μg estradiol, and 250 μg testosterone. The estradiol and testosterone were in the form of a water-soluble complex with cyclodextrin. Blood samples were collected prior to and after dosing on each test day for quantitation of serum estradiol and testosterone levels. The results are presented in Figs. 1A and 1B.

[00120] Fig. 1A is a bar graph showing the cumulative area under the curve (AUC) from 0 to 360 minutes for serum estradiol, in pg*min/mL, for each of the six patients. Fig. 1B is a similar graph for testosterone. The hormone levels on day 1 and day 8, corresponding to dose 1 and dose 2, are shown as a separate bar for each patient. Comparison of the AUC of each patient shows that uptake of the compounds in the nasal preparation is relatively uniform, with variations between patients likely due to varying extent of metabolic conversion during nasal mucosa absorption. Importantly, the data also show that significant absorption of estradiol and testosterone occurs in the presence of the GnRH compound.

[00121] In this study, the estrogenic compound estradiol and testosterone were present in the formulation at a molar ratio of 1:5 (MW estradiol = 272.4 g/mol; MW testosterone = 288.4 g/mol). The ratio of these two hormones can range from between about 1:1 to 1:5, and more preferably range from about 1:1 to 1:3, and most preferably from 1:1 to 1:2.

[00122] Example 4 describes a study performed in support of the invention where the nasal preparation is similar to that of Example 1, comprised of a GnRH compound, estradiol, and testosterone, was administered to healthy, premenopausal women. Three doses of the GnRH compound deslorelin were tested, 0.5 mg, 1.0 mg, and 2.0 mg. The nasal preparation was administered using a conventional metered nasal spray delivery device. The subjects received two 50 μL sprays, one in each nostril, daily for four weeks. Blood samples were collected prior to drug administration on day 1 of the study, and then at regular intervals throughout day 1. Thereafter, blood samples were collected weekly, until day 29, when blood was collected at scheduled intervals throughout the day. Serum deslorelin, estradiol, testosterone, and progesterone were quantified, and the results are shown in Tables 6A through 6C, below in Example 4.

[00123] The degree of induced ovarian suppression is evident from the serum estradiol and progesterone levels (Tables 6A - 6C). Serum levels of estradiol on day 29 varied from 14 to 103 pg/mL. Progesterone levels were generally less than 80 ng/dL during the treatment interval indicating that women were anovulatory during the treatment.

[00124] In one embodiment, the intranasal dose of the estrogenic compound, and the optional androgen if present, achieve a transient serum level outside the serum estradiol level of between about 25 pg/mL to about 140 pg/mL that is typically reported in the literature with a 50 µg/day transdermal patch. Although the serum hormone levels resulting from intranasal delivery of the hormone(s) are transiently outside this range a similar beneficial effect is achieved. That is, the biological effect(s) resulting from intranasal delivery of an estrogenic compound, and the optional androgenic compound, is similar to the biological effect associated with a serum estradiol level of between about 25 pg/mL to about 140 pg/mL even though the actual transient serum level may be outside this range. Thus, in one embodiment, the invention contemplates administration of an estrogenic compound and an optional androgenic compound in an amount sufficient to achieve the beneficial biological effects that are associated with an steady estradiol serum level of between about 25 pg/mL to about 140 pg/mL, more preferably between about 30 pg/mL to about 100 pg/mL, most preferably between about 30 pg/mL to about 50 pg/mL. In intranasal formulations where the optional androgenic compound is present, the transient androgen serum blood level achieved may be lower or higher than that typically obtained by other routes of administration. However, the beneficial effects achieved by intranasal administration are similar to those obtained from a steady serum testosterone level of between about 20 ng/dL to about 80 ng/dL, more preferably between about 40 ng/dL to about 60 ng/dL.

[00125] A comparison of the total area under concentration-time curves (AUC) or average concentrations of serum estradiol (or testosterone) in subjects treated with intranasal estradiol (or testosterone) and subjects treated with estradiol (or testosterone) by another route, such as transdermal, provides a basis for determining the biological equivalency of different routes of administration. Where the AUCs or average concentrations are similar, despite different routes of administration or different concentration-time profiles, the biological effect achieved is often similar. Thus, in one embodiment, the invention contemplates achieving by intranasal administration of the disclosed composition an average serum estradiol concentration over 24 hours of between about 25 pg/mL and about 50 pg/mL. In nasal preparations containing the optional androgenic compound testosterone, the invention contemplates achieving an average serum testosterone concentration over 24 hours of between about 15 ng/dL and about 40 ng/dL.

[00126] The incidence of development of simple endometrial hyperplasia resulting from co-administration of a GnRH compound and unopposed estradiol was evaluated in

a study described in Example 7. Two-hundred sixty-five premenopausal women participated in a year long study where deslorelin was administered daily as a nasal spray and estradiol was administered in the form of a transdermal patch. At the end of the year endometrial biopsies were evaluated for hyperplasia. The results, which are shown in Table 8 (see Example 7 below), show that the incidence of simple hyperplasia for untreated subjects (Arm 1, placebo/placebo) was 2.2%. The incidence of simple hyperplasia for subjects treated with intranasal deslorelin and transdermal estradiol (Arms 3, 4 and 5) was 0% (Arms 3 and 5) or 4.2% (Arm 4). The incidence in combined Arms 4 and 5 was 2.0%. These data show that delivery of a GnRH compound with unopposed estrogen (that is, estrogen in the absence of a progestin) resulted in little risk of endometrial hyperplasia, with the risk no greater than that of the women in the untreated population (Arm 1). The patients in Arm 2 of the study, where estradiol was absent for the first 6 months of the study and then added for the final 6months, had a 16.7% incidence of simple hyperplasia.

[00127] In a similar study, described in Example 6, twenty women were treated with deslorelin and unopposed estradiol. The women were divided into treatment groups to receive intranasal deslorelin and intranasal estradiol (Arm 1), intranasal deslorelin and transdermal estradiol (Arm 2), or intranasal deslorelin and intranasal estradiol plus testosterone (Arm 3). At the end of the6 month treatment period, the endometrial response was evaluated by biopsy.

[00128] The results of the biopsies are shown in Table 7, presented below in Example 6. The endometrial tissue was proliferative in the 16 evaluable biopsies, and there was no evidence of simple endometrial hyperplasia in any of these evaluable subjects after 6 months of treatment.

[00129] The studies described in Examples 6 and 7 show that women administered a GnRH compound and an estrogen, and optionally an androgen, by non-gastrointestinal routes, and preferably intranasally or transdermally, are not at increased risk of endometrial simple hyperplasia. The data show that a GnRH compound and an estrogenic compound can be administered to premenopausal women with no increased risk of developing simple endometrial hyperplasia relative to women receiving placebo. This observation is unexpected since typically 30% of postmenopausal women treated with unopposed estrogen (0.625 mg dose) develop simple hyperplasia (Gefland, M. *et al. Obstetrics & Gynecology*, 74:398 (1989); *JAMA*, 275(5):370 (1996); Clisham, P. *et al., Obstetrics & Gynecology*, 79:196 (1992)). Premenopausal women treated with a GnRH compound and an estrogen were expected to be similar to postmenopausal women, since the GnRH

compound reduces serum estradiol and serum progesterone levels. However, the data clearly demonstrated that premenopausal women treated with a GnRH compound and an estrogenic compound for an extended time period (e.g., up to one year) had no increased incidence of simple hyperplasia. Thus, addition of a progestin to the treatment regimen of a GnRH compound and an estrogenic compound in premenopausal women was not and is not needed to protect against simple endometrial hyperplasia. This observation is particularly seen in women who have no prior estrogen deprivation (compare Arms 3, 4, 5 with Arm 2 in the study describe in Example 7). The studies further suggest that such treatment can continue for a period of about 6 to 12 months or longer with no significant risk of developing simple hyperplasia.

[00130] Based on these studies, the phrase "no significant risk" as used herein intends that fewer than about 10%, and more preferably less than 5%, still more preferably less than about 2% of premenopausal women treated with a GnRH compound and an estrogenic compound are at risk of developing simple endometrial hyperplasia. In summary, the studies show that treatment of benign gynecological disorders with a GnRH compound and an estrogenic compound and, optionally, an androgenic compound, or contraception with the two or three compounds, need not be accompanied by treatment with a progestin on a regular or periodic basis in order to protect against simple endometrial hyperplasia or cancer.

[00131] In a study performed in support of the invention, the average serum concentration over 24 hours resulting from transdermal administration of estradiol and from intranasal administration of estradiol were compared. As described in Example 10, test subjects received estradiol transdermally or intranasally. Transdermal estradiol was administered using a Noven Vivelle® or a Noven Vivelle-dot® transdermal patch, both at dosages of 50 μg/day. The subjects treated intranasally received 350 μg estradiol in a liquid spray delivered once per day. Average concentrations for each of the patient populations were determined from blood samples, and the results are summarized in Table 11 below in Example 10.

[00132] The average serum estradiol concentration over 24 hours for women receiving estradiol transdermally from the Vivelle® patch was 34.4 pg/mL and from the Vivelle-dot® patch was 36.8 pg/mL. The average estradiol concentration for women treated with intranasal estradiol was 37.8 pg/mL. This study shows that an estrogenic compound administered as an intranasal bolus achieves a 24 hour average serum concentration comparable to that achieved by transdermal administration. Thus, in one embodiment the invention provides a 24 hour average serum concentration of estradiol from an intranasal

bolus dose of estradiol that is within (plus or minus) about 10% of the 24 hour average estradiol serum concentration achieved from transdermal estradiol administration. That is, the 24 hour average estradiol serum concentration from intranasal bolus administration of estradiol is at least about 90% of the 24 hour average estradiol serum concentration from transdermal administration of estradiol. This result was surprising since heretofore it was unknown (i) if a bolus dose would achieve efficacious blood concentration and (ii) if a bolus dose would achieve a concentration comparable to that of a controlled-release transdermal dose. The data shows that an intranasal bolus dose of an estrogenic compound achieves a therapeutic blood concentration, and that the concentration is comparable, *i.e.*, is within at least about 10%, to that achieved by transdermal administration of the estrogenic compound.

[00133] In another study, five women were treated with intranasal testosterone. In this study, 250 μg testosterone was formulated into a nasal preparation also containing deslorelin and estradiol (Example 1). The formulation was administered initially on day 1 of the study, and then again one week later on day 8 of the study. The average serum concentrations over 24 hours are shown in Table 3.

Table 3: Testosterone: Average Concentration over 24 Hours after Intranasal Administration of 250 μg

Subject Number	Treatment Day	Testosterone (average concentration over 24 hours, ng/dL)
1	Day 1	26.7
	Day 8	24.1
2	Day 1	26.7
	Day 8	29.9
3	Day 1	19.0
	Day 8	17.1
4	Day 1	18.8
	Day 8	16.5
5	Day 1	19.1
	Day 8	16.2
Average of both observations in each of the 5 subjects		21.4

[00134] The average concentration over 24 hours for both doses in the five subjects

was 21.4 ng/dL. This concentration is comparable to literature reported values achieved from transdermal administration of testosterone. For example, in Javanbakht*et al.* (*J. of Clinical Endocrinology and Metabolism,* 85(7): 2935, 2000) women wearing a transdermal testosterone patch for 96 hours that delivered 300 μg/day had an average serum concentration of 15.8 ng/dL. Thus, the nasal formulation of the present invention,*i.e.,* a bolus dose of testosterone, provides a similar area under the curve as a slow-release transdermal formulation, as evidenced by similar average concentration values.

[00135] In one embodiment, the intranasal dose of the estrogenic compound achieves a transient serum level outside the serum estradiol level of between about 25 pg/mL to about 140 pg/mL that is typically reported in the literature with a 50 μg/day transdermal patch. Although the serum hormone level resulting from intranasal delivery of the hormone is transiently outside this range a similar beneficial effect is achieved. That is, the biological effect(s) resulting from intranasal delivery of an estrogenic compound is similar to the biological effect associated with a serum estradiol level of between about 25 pg/mL to 140 pg/mL even though the actual transient serum level may be outside this range. Thus, in one embodiment, the invention contemplates administration of an estrogenic compound in an amount sufficient to achieve the beneficial biological effects that are associated with a steady estradiol serum level of between about 25 pg/mL to 140 pg/mL, more preferably between about 30 pg/mL to about 100 pg/mL, most preferably, between about 30 pg/mL to about 50 pg/mL.

[00136] Similarly, the transient testosterone serum blood level achieved may be lower or higher than that typically obtained by other routes of administration. However, the physiological beneficial effects achieved by intranasal administration are similar to those obtained from a serum testosterone level of between about 15 ng/dL to about 80 ng/dL, more preferably between about 40 ng/dL to 60 ng/dL.

[00137] A comparison of the total area under concentration-time curves (AUC) or average concentrations of serum estradiol and testosterone in subjects treated with intranasal estradiol and testosterone to patients treated with estradiol and testosterone by another route, such as transdermal, provides a basis for determining the biological equivalency of different routes of administration. Where the AUCs or average concentrations are similar, despite different routes of administration or different concentration-time profiles, the biological effect achieved is often similar. Thus, in one embodiment, the invention contemplates achieving by intranasal administration of the disclosed composition an average serum estradiol concentration over 24 hours of between about 25 pg/mL and about 50 pg/mL. The invention further contemplates

achieving an average serum testosterone concentration over 24 hours of between about 15 ng/dL and about 40 ng/dL.

[00138] Example 9 describes a study performed in support of the invention where the efficacy and biologic equivalence between nasal spray add-back estradiol and transdermal estradiol add-back were evaluated. Women with endometriosis treated with intranasal deslorelin (GnRH compound) were assigned to one of three methods of estradiol add-back: (1) 50 µg/day estradiol transdermal patch, (2) 300 µg/day intranasal estradiol, or (3) 300 µg/day intranasal estradiol with 275 µg/day intranasal testosterone. Treatment efficacy was measured by evaluating the decrease in endometriosis using a standard scoring system that takes into account 3 symptoms (pelvic pain, dysmenorrhea, and dyspareunia) and two signs (pelvic tenderness and pelvic induration) of endometrosis. The results are shown in Table 10A in Example 9 as the composite score physical symptoms and signs score (CPSSS) taken as the sum of the scores for each individual symptom or sign (0 to 3 with 0 being not present and 3 being the most severe). The nasal spray preparations with at least 90% of the estradiol average serum concentration (AUC) of the transdermal patch (Table 11) were more effective than the transdermal patch.

[00139] Loss of bone mineral density (BMD) is a known side effect of treatment with GnRH compounds. Thus, BMD of the lumbar spine of the test subjects in Example 9 was obtained by dual-energy X-ray absorptiometry (DEXA) prior to and after six months of treatment. The results are shown in Table 10B in Example 9 as the ratio of BMD at thesix month time point ($BMD_{6mo.}$) to the BMD prior to treatment ($BMD_{baseline}$). BMD of subjects treated with deslorelin alone in another study (Example 8) is also shown in Table 10B for comparison. A reduction in loss of BMD by addition of estradiol to deslorelin is apparent, since all subjects treated with estradiol had reduced bone loss. The data also shows that estradiol add-back in the form of an intranasal bolus is at least as effective, and in fact slightly more effective, in preventing loss of BMD than a transdermal estradiol add-back, as observed by comparing the BMD ratios for women receiving estradiol transdermally ($BMD_{6mo.}$/ $BMD_{baseline}$ = 0.978) and for women receiving estradiol intranasally ($BMD_{6mo.}$/ $BMD_{baseline}$ = 0.996).

[00140] The data in Tables 10A-10B demonstrate that the estradiol added-back in the form of an intranasal bolus dose to deslorelin resulted in a significant decrease in endometriosis symptoms and at the same time reduced the loss of BMD. A nasal spray preparation comprised of a GnRH compound and an estrogenic compound, where the preparation has an 24 hour average estrogenic compound serum concentration (AUC) within 10% of the 24 hour average transdermal estrogenic compound serum concentration

(Table 11), was at least as effective, and preferably more effective, in preventing loss of BMD than an add-back estrogenic compound in the form of a transdermal patch.

[00141] From the foregoing, it can be seen how various objects and features of the invention are met. Treatment of benign gynecological disorders and contraception by delivery of a GnRH compound, an estrogenic compound, and optionally an androgenic compound, to premenopausal women not receiving an exogenously supplied progestin on a regular or periodic basis did not increase the risk of simple endometrial hyperplasia relative to women receiving placebo.

[00142] It can also be seen how contraception and/or treatment of benign gynecological disorders by intranasal delivery of a GnRH compound and an estrogenic compound effectively suppresses ovarian estrogen and progesterone production. The nasal preparations described herein contain the estrogenic compound in the form of a water-soluble complex with a water-soluble cyclodextrin. In some embodiments, a second steroid, such as testosterone or a progestin, is included. Absorption of the GnRH compound in the presence of the steroids is adequate to achieve suppression of ovarian estrogen and progesterone production. Further, the nasal formulations tested were non-irritating to the test subjects.

[00143] It can further be seen it can be seen how contraception and treatment of benign gynecologic disorders, both in conjunction with a GnRH compound, can be complimented by administration of add-back steroids delivered in the form of an intranasal preparation. Hormone replacement therapy can also be achieved by intranasal delivery of steroids. More specifically, an estrogenic compound and an androgenic compound and, optionally a progestin compound, are complexed with cyclodextrin to form a water-soluble complex of the two or three steroids in the cyclodextrin. The preparation when administered intranasally has minimal, if any, nasal irritation, and achieves a 24 hour serum steroid concentration that is within about 10% of the 24 hour serum steroid concentration observed from transdermal administration of the steroids.

[00144] The studies described herein also showed that the nasal preparation, when administered to premenopausal women not receiving exogenously supplied progesterone, did not increase the risk of simple endometrial hyperplasia, relative to women receiving placebo. The studies suggest that such treatment can continue for a period of 6 to 12 months or longer with no significant risk of simple endometrial hyperplasia.

III. Examples

[00145] The following examples further illustrate the invention described herein and are

in no way intended to limit the scope of the invention.

Example 1

Preparation of Intranasal Formulation

[00146] 2-Hydroxypropyl-β-cyclodextrin was added to water at a concentration of 240 mg/mL and stirred until dissolved. 17ß-Estradiol was then added to the water-cyclodextrin solution at a concentration of 1.0 mg/mL. The mixture was stirred until dissolution. Testosterone at a concentration of 5.0 mg/mL was then added, and after stirring to dissolution benzalkonium chloride (0.1 mg/mL), ethylene diamine tetra acetic acid (EDTA 1 mg/mL), and sorbitol (61.6 mg/mL) were added. The mixture was stirred. Then, the GnRH compound deslorelin acetate was added at a concentration of 20 mg/mL with stirring. The volume was brought to the final desired volume and the pH was adjusted as needed. Tables 4A and 4B summarizes the preparation components, concentrations, and dosages per 50 µL.

Table 4A: Components in Exemplary Nasal Preparation

Component	Concentration (mg/mL)	Dose per 50 µL
deslorelin acetate	20	1.0 mg
estradiol	1.0	50 µg
testosterone	5.0	250 µg
hydroxypropyl β cyclodextrin	240	12 µg
benzalkonium chloride	0.1	5 µg
EDTA	1.0	50 µg
sorbitol	61.6	3.1 mg
water, USP	as required	

Table 4B: Components in Exemplary Steroid Nasal Preparation

Component	Concentration (mg/mL)	Dose per 50 µL
17ß-estradiol	1.0	50 µg
Testosterone	5.0	250 µg
2-hydroxypropyl-ß-cyclodextrin	240	12 µg
Benzalkonium chloride	0.1	5 µg
EDTA	1.0	50 µg
Sorbitol	61.6	3.1 mg
Water, USP	as required	

Example 2

Intranasal Administration of Deslorelin to Premenopausal Women with Uterine Leiomyomata

[00147] A 12 week study was performed to establish an effective dose of deslorelin for controlling heavy bleeding secondary to uterine leiomyomata. Forty-one women completed the study and are identified as Subject Nos. 1-41. The women were divided into test groups for treatment with intranasal deslorelin as follows:

Group 1	Subject Nos. 1-6	placebo, 0 mg deslorelin
Group 2	Subject Nos. 7-21	0.5 mg deslorelin, once per day
Group 3	Subject Nos. 22-34	1.0 mg deslorelin, once per day
Group 4	Subject Nos. 35-41	2.0 mg deslorelin, once per day

[00148] An intranasal preparation consisting of deslorelin at the indicated concentration along with sorbitol (61.6 mg/mL), benzalkonium chloride (0.1 mg/mL), and water was prepared according to the procedure of Example 1. The preparation was administered with a commercially available nasal sprayer that delivered a 50 μL spray volume. The preparation was administered by application of 50 μL to each nostril once per day, for a total daily spray volume of 100 μL to give the indicated dose of deslorelin.

[00149] The average age of the premenopausal patients was 42.3, with similar distribution among groups. For one complete menstrual cycle prior to treatment, each woman completed a daily bleeding calendar. Eligible subjects were then treated with deslorelin at the assigned dosage once per day by intranasal application. During the 12 weeks of daily intranasal administration, each woman kept a daily bleeding calendar, completed quality of life questionnaires, and underwent clinical assessment and laboratory testing. The subjects were tracked for 6 weeks post-treatment for further assessment and to document time to recovery of menses after last drug treatment day. Clinical assessments included grading of nasal irritation (Table 5A), determination of uterine size (Table 5B), and serum hormone levels (Table 5C). The bleeding scores are presented in Table 5D.

Table 5A: Nasal Irritation

		Number of Study Subjects			
Deslorelin (mg/day)		0 mg	0.5 mg	1 mg	2 mg
Baseline	None	11	11	13	9
	Slight	2	3	0	2
	Moderate	0	0	1	3
	Quite a bit	0	0	0	0
	Extreme	0	0	0	0
	Total	13	14	14	14
End of Week 4					
	None	13	11	13	12
	Slight	0	2	0	1
	Moderate	0	1	1	0
	Quite a bit	0	0	0	1
	Extreme	0	0	0	0
	Total	13	14	14	14
End of Week 8					
	None	13	11	13	11
	Slight	0	1	1	3
	Moderate	0	1	0	0
	Quite a bit	0	1	0	0
	Extreme	0	0	0	0
	Total	13	14	14	14
End of Week 12					
	None	13	11	13	8
	Slight	0	2	0	5
	Moderate	0	0	0	1
	Quite a bit	0	1	1	0
	Extreme	0	0	0	0
	Total	13	14	14	14

Table 5B: Uterine Size

Deslorelin (mg/day)	Mean Difference*
0 mg	+11.6
0.5 mg	-109.6
1 mg	-63.6
2 mg	-246.5

*end of week 12 minus baseline uterine volume (cm^3)

Table 5C: Hormone Levels

	Estradiol (pg/mL)	Progesterone (ng/dL)	Testosterone (ng/dL)
Baseline			
0 mg	164	560	32
0.5 mg	173	641	24
1 mg	123	480	26
2 mg	184	337	34
End of Week 4			
0 mg	141	121	27
0.5 mg	47	40	19
1 mg	51	12	22
2 mg	14	12	16
End of Week 8			
0 mg	134	142	31
0.5 mg	79	30	22
1 mg	95	25	20
2 mg	31	14	20
End of Week 12			
0 mg	152	382	29
0.5 mg	66	37	23
1 mg	30	10	16
2 mg	30	13	20

Table 5D: Bleeding Score*

	Deslorelin (mg/day)			
	0 mg	0.5 mg	1 mg	2 mg
Baseline Score*	10.2	10.9	10.8	14.1
Weeks 1-4	9.5	6.6	7.8	6.6
Weeks 5-8	8.4	3.5	0.4	1.2
Weeks 9-12	5.2**	2.6	1.7	0.6

*Bleeding scores are calculated from the sum of daily diary entries for the 28 day interval prior to the reporting period. No bleeding throughout the interval is a score of 0, 'normal' menstrual flow is a score of 5, and menorrhagia is a score of 10 or greater.

**25% of subjects in the placebo group dropped out of the study and a large placebo effect was observed in some of the subjects remaining on study.

Example 3

Intranasal Administration of a GnRH compound, an Estrogen, and an Androgen to Oophorectomized Women

[00150] Six volunteer women with prior oophorectomies and not presently on hormone replacement therapy were recruited. Each woman was treated with 50μL of a nasal spray preparation, prepared as described in Example 1, on two occasions separated by one week. The 50 μL dose delivered 1 mg deslorelin, 50 μg 17ß-estradiol, and 250 μg testosterone. Blood samples were collected 20 minutes and 10 minutes prior to dosing on day 1 and on day 8, and then at the following intervals after dosing on each day: 10, 20, 30, 40, 60, 90, 120, 180, 240, 360, 1440 minutes. Serum estradiol and testosterone levels

were determined from the samples, and the baseline corrected cumulative area under the curve from 0 to 360 minutes for each patient for each dose are presented in Figs. 1A and 1B.

Example 4

Intranasal Administration of Deslorelin, Estradiol, and Testosterone to Premenopausal Women

[00151] Nine premenopausal women, ages 20 to 45 years, were recruited and randomly divided into three test groups for a 29 day study. The patients in Group 1, Group 2, and Group 3 were treated with the intranasal preparation similar to that described in Example 1 but with deslorelin acetate concentrations of 5 mg/mL (Group 1), 10 mg/mL (Group 2), or 20 mg/mL (Group 3). The single intranasal administration consisted of a 100µL dose delivered using a metered nasal spray device as two 50 µL sprays, one in each nostril.

[00152] An indwelling intravenous catheter was inserted in an arm vein for withdrawal of blood samples prior to drug administration and at defined intervals post administration (study day 1) of 40, 120, 240, and 480 minutes. Thereafter, weekly blood samples (study days 8, 15, and 22) were collected for determination of serum estradiol, progesterone, testosterone, and deslorelin levels. On study day 29 post administration, blood samples were drawn according to the same regimen on study day 1. After collection, all blood samples were allowed to clot at room temperature, then refrigerated. Within 24 hours of collection, serum was separated and stored frozen at –5°C until assayed.

[00153] Serum levels of estradiol, testosterone, and progesterone were quantitated by sensitive and specific radioimmunoassay methods (Stanczyk, F.Z. *et al.*, *Am. J Obstet. Gynecol.*, 159(6):1540 (1988); Scott *et al.*, *Am. J Obstet. Gynecol.*, 130(7):817 (1978)). Prior to assay of the steroid hormones, serum was extracted with ethyl acetate:hexane (1:1) and for the testosterone assay further purified via Celite™ column chromatography, with 40% toluene to elute the testosterone. Procedural losses were followed by addition of 1000 dpm of the appropriate tritiated internal standard. The sensitivities of the estradiol, testosterone, and progesterone assays were 8 pg/mL, 4 ng/dL, and 10 ng/dL, respectively. Assay accuracy was demonstrated by observed parallelism between standard curves and serially diluted serum with respect to each hormone. Intra- and inter-assay coefficients of variation were 5% to 10% and 10% to 15%, respectively. Specificity of the assays was enhanced by eliminating interfering metabolites with extraction and/or chromatography and through the use of highly specific antisera.

[00154] The results of all hormone analyses are presented for each subject in Tables 6A to 6C.

Table 6A: Hormone Levels for Patients Treated with 0.5 mg/mL Deslorelin

Study Subject*	Study Day	Time (min)	Estradiol pg/mL	Testosterone ng/dL	Progesterone ng/dL
#1	1	0	61	31	110
		40	91	142	
		120			
		240			
		480			
	8		159	29	90
	15		300	28	100
	22		10	15	40
	29	0	14	13	100
		40	57	137	
		120			
		240			
		480			
	42				1260
#2	1	0	33	18	100
		40	42	43	
		120			
		240			
		480			
	8		130	25	50
	15		160	25	40
	22		39	19	50
	29	0	52	18	60
		40	50	20	
		120			
		240			
		480			
	42				40
#3	1	0	54	21	90
		40	128	190	
		120			
		240			
		480			
	8		85	39	110
	15		98	36	
	22		37	22	40
	29	0	103	86	70
		40	112	142	
		120			
		240			
		480			
	42				

*Treated with the nasal preparation of Example 1 having 0.5 mg/mL deslorelin acetate.

Table 6B: Hormone Levels for Patients Treated with 1.0 mg/mL Deslorelin

Study Subject*	Study Day	Time (min)	Estradiol pg/mL	Testosterone ng/dL	Progesterone ng/dL
#4	1	0	73	21	40
		40	98	81	
		120			
		240			
		480			
	8		167	31	50
	15		91	33	50
	22		86	24	50
	29	0	40	23	60
		40	126	144	
		120			
		240			
		480			
	42				
#5	1	0	73	21	220
		40	227	250	
		120			
		240			
		480			
	8		26	19	50
	15		27	23	30
	22		132	37	50
	29	0	45	25	40
		40	98	102	
		120			
		240			
		480			
	42				
#6	1	0	51	20	60
		40	181	174	
		120			
		240			
		480			
	8		45	26	80
	15		18	20	70
	22		48	16	60
	29	0	29	20	100
		40	89	98	
		120			
		240			
		480			
	42				

* Treated with the nasal preparation of Example 1 having 1.0 mg/mL deslorelin acetate.

Table 6C: Hormone Levels for Patients Treated with 2.0 mg/mL Deslorelin

Study Subject*	Study Day	Time (min)	Estradiol pg/mL	Testosterone ng/dL	Progesterone ng/dL
#7	1	0	32	14	90
		40	141	149	
		120			
		240			
		480			
	8		21	13	50
	15		16	14	60
	22		12	9	40
	29	0	23	10	70
		40	40	24	
		120			
		240			
		480			
	42				
#8	1	0	61	15	80
		40	191	244	
		120			
		240			
		480			
	8		149	30	50
	15		302	39	70
	22		22	22	150
	29	0	90	25	70
		40	117	88	
		120			
		240			
		480			
	42				
#9	1	0	37	21	30
		40	152	241	
		120			
		240			
		480			
	8		158	110	20
	15		18	18	20
	22		24	15	10
	29	0	29	14	20
		40	143	132	
		120			
		240			
		480			
	42				

* Treated with the nasal preparation of Example 1 having 2.0 mg/mL deslorelin acetate.

Example 5

Solubility of Estradiol and Testosterone in Cyclodextrin/Water

[00155] The solubility of 17-ß-estradiol and testosterone in varying concentrations of 2-hydroxypropyl-β-cyclodextrin (MW 1380 g/mole; 5.5 degree of substitution) was

determined as follows. 10 ng 17-ß-estradiol (MW 272.39 g/mole) was added to 1 mL of 2-hydroxypropyl-β-cyclodextrin in water, the 2-hydroxypropyl-β-cyclodextrin concentration ranging from 10 to 250 ng/mL. In a second series of vials, 20 ng of testosterone (MW 288.43 g/mole) was added to 1 mL of 2-hydroxypropyl-β-cyclodextrin in water, the 2-hydroxypropyl-β-cyclodextrin concentration ranging from 10 to 250 ng/mL. In a third set of vials 10 ng 17-ß-estradiol and 20 ng testosterone were added to 1 mL of 2-hydroxypropyl-β-cyclodextrin in water, the 2-hydroxypropyl-β-cyclodextrin concentration ranging from 10 to 250 ng/mL. The vials were mixed at room temperature for about 1 hour. Aliquots were taken from the supernatant of each vial and assayed for steroid concentration. The results are shown in Tables 2A and 2B.

Example 6

Intranasal Delivery of GnRH Compound with Transdermal or Intranasal Co-administration of Estradiol

[00156] Twenty premenopausal women were recruited and randomly assigned for treatment as follows:

Arm 1	deslorelin, intranasal and estradiol transdermal	n=5
Arm 2	deslorelin and estradiol, intranasal	n=7
Arm 3	deslorelin, estradiol, and testosterone intranasal	n=8

[00157] The Arm 1 intranasal formulation contained 1 mg deslorelin; the Arm 1 estradiol was delivered transdermally from a twice-weekly commercially-available 50 µg/day estradiol patch. The intranasal formulation used in Arms 2 and 3 contained 1 mg deslorelin and 300 µg estradiol (Arm 2), and additionally 275 µg testosterone (Arm 3), formulated in a similar manner as that described in Example 1.

[00158] After the six month treatment period the incidence of endometrial hyperplasia was evaluated by biopsy in 20 subjects. The results are shown in Table 7.

Table 7: Endometrial Response

	Hyperplasia	Proliferative	Atrophic	Insufficient Tissue	Refused
Baseline	0	17	0	3	0
Month 6	0	16	0	3	1

[00159] Although the invention has been described with respect to particular embodiments, it will be apparent to those skilled in the art that various changes and

modifications can be made without departing from the invention.

Example 7

Intranasal Delivery of GnRH Compound with Transdermal Co-administration of Estradiol

[00160] Example 7 describes an intranasally delivered GnRH compound with co-administration of 17ß-estradiol. Premenopausal females (n=265) were recruited for participation in a 12 month double blind study. The women were randomly assigned to treatment in one of the following five study arms:

Arm 1 placebo/placebo

Arm 2 deslorelin, intranasal/placebo for 6 months; then crossed over to arm 5

Arm 3 deslorelin, intranasal/25 μg estradiol transdermal

Arm 4 deslorelin, intranasal/50 μg estradiol transdermal

Arm 5 deslorelin, intranasal/75 μg estradiol transdermal

[00161] Deslorelin at a daily dose of 1 mg was administered intranasally using a conventional metered delivery device. Estradiol was administered transdermally using a commercially-available twice-weekly patch that delivered either 25 μg estradiol or 50 μg estradiol per day. Subjects in Arm 5 wore two patches, one at each dosage, to achieve the 75 μg dose.

[00162] At the end of the 12 month treatment period an endometrial biopsy was taken for analysis of the endometrial morphology. The results are shown in Table 8.

Table 8: Incidence of Simple Hyperplasia from Endometrial Biopsy

	Arm					
	1	2	3	4	5	4&5
No. of subjects with simple hyperplasia	1	2	0	1	0	1
Total no. of subjects studied	45	12	6	24	25	49
Proportion of subjects with simple hyperplasia	0.022	0.167	0.000	0.042	0.000	0.020

Example 8

Intranasal Delivery of GnRH Compound with Transdermal Co-administration of Estradiol

[00163] Example 8 describes an intranasally delivered GnRH compound with co-administration of estradiol. Premenopausal females (n=265) with uterine fibroids were recruited for participation in a 12 month double blind study. The women were randomly

assigned to treatment in one of the following five study arms:

Arm 1 placebo/placebo

Arm 2 deslorelin, intranasal/placebo for 6 months; then crossed over to arm 5

Arm 3 deslorelin, intranasal/25 μg estradiol, transdermal

Arm 4 deslorelin, intranasal/50 μg estradiol, transdermal

Arm 5 deslorelin, intranasal/75 μg estradiol, transdermal

[00164] Deslorelin at a daily dose of 1 mg was administered intranasally using a conventional metered spray delivery device. The intranasal preparation was a 100 μL daily nasal spray containing 1.0 mg deslorelin. Estradiol was administered transdermally using a commercially available twice-weekly patch that delivered either 25 μg estradiol or 50 μg estradiol per day. Subjects in Arm 5 wore two patches, one at each dosage, to achieve the 75 μg dose.

[00165] Women with uterine fibroids often experience very heavy bleeding (menorrhagia) and uterine enlargement leading to pelvic pain and pressure symptoms. Clinical benefit was assessed by measuring changes in bleeding score (percent of subjects with reduction of bleeding into the normal range) and uterine volume. Shown in Table 9A are the percent of subjects responding (reduction of bleeding into the normal range) after 3 and 6 months of treatment. The percent of subjects having a response is significantly greater in the active treatment groups compared to the placebo group. Table 9B shows the change in uterine volume (expressed as proportion of initial volume) associated with treatment. The placebo group had an increase in uterine volume and active treatment resulted in decreased uterine volume.

Table 9A: Percent of Subjects with Reduction of Bleeding into the Normal Range

	3 Months		6 Months	
	No. of Subjects	% with reduction	No. of subjects	% with reduction
Placebo	79	10	70	17
Deslorelin	36	69	36	92
Deslorelin + 25 μg/day transdermal estradiol	32	75	27	85
Deslorelin + 50 μg/day transdermal estradiol	37	73	35	66
Deslorelin + 75 μg/day transdermal estradiol	42	81	38	68

Table 9B: Proportion of Uterine Volume: Volume after 6 Months of Treatment Compared to Baseline

	6 Months	
	No. of subjects	Proportion of Initial Volume
Placebo	60	1.238
Deslorelin	33	0.675
Deslorelin + 25 µg/day transdermal estradiol	24	0.805
Deslorelin + 50 µg/day transdermal estradiol	31	0.789
Deslorelin + 75 µg/day transdermal estradiol	37	0.910

Example 9

Intranasal Delivery of GnRH Compound with Transdermal or Intranasal Co-administration of Estradiol

[00166] Women with endometriosis treated with intranasal deslorelin (GnRH compound) were assigned to one of three methods of add-back: 1) 50 µg/day estradiol transdermal patch, 2) 300 µg/day intranasal estradiol, or 3) 300 µg/day intranasal estradiol with 275 µg/day intranasal testosterone. Treatment efficacy was measured by evaluating the decrease in endometriosis symptoms associated with treatment. Evaluated were symptoms and signs of endometriosis using a standard scoring system that takes into account 3 symptoms (pelvic pain, dysmenorrhea, and dyspareunia) and two signs (pelvic tenderness and pelvic induration). The composite score physical symptoms and signs score (CPSSS) is the sum of the scores for each individual symptom or sign (0 to 3 with 0 being not present and 3 being the most severe). Shown in Table 10A is the change in CPSSS following 3 and 6 months of treatment.

Table 10A: CPSSS after 3 and 6 Months of Drug Treatment

	Baseline CPSSS	Month 3	Month 6	No. of subjects
Deslorelin + Transdermal Estradiol	7.4	4.0	3.8	5
Deslorelin + Intranasal Estradiol	8.1	2.3	3.4	7
Deslorelin + Intranasal Estradiol + Testosterone	6.8	1.6	3.1	8

[00167] Bone mineral density (BMD) of the lumbar spine was obtained by dual-energy X-ray absorptiometry (DEXA) prior to and after six months of drug treatment. BMD

changes are shown in Table 10B as the ratio of the 6 month value compared to the baseline value. BMD of subjects treated with deslorelin alone (Example 8) was 0.971 of the baseline value.

Table 10B: Effect of Add-back Estradiol on the Ratio of 6 Month BMD to Baseline BMD

	Mean Ratio ($BMD_{6mo}/BMD_{baseline}$)	No. of subjects
Deslorelin*	0.971	36
Deslorelin + Transdermal Estradiol	0.978	5
Deslorelin + Intranasal Estradiol	0.996	7
Deslorelin + Intranasal Estradiol + Testosterone	0.999	8

*From study described in Example 8.

Example 10

Comparison of Intranasally and Transdermally Delivered Estradiol

[00168] Naturally postmenopausal or surgically postmenopausal females (n=63) were recruited for the study. Thirty women were selected for treatment with transdermal estradiol from a Noven Vivelle® 50 μg/day patch. Thirty women were treated with transdermal estradiol from a Noven Vivelle-dot® 50 μg/day patch. The remaining three women were treated with a single bolus, 100 μL volume, nasal spray containing 350 μg 17-ß-estradiol per 100 μL bolus. The nasal formulation in addition to estradiol was comprised of sorbitol (61.6 mg/mL), EDTA (1.0 mg/mL), benzalkonium chloride (0.1 mg/mL), and 2-hydroxypropyl-β-cyclodextrin (100 mg/mL).

[00169] Blood samples were drawn at defined intervals for analysis of serum estradiol levels. The average concentration over 24 hours as pg/mL was determined and the results are shown in Table 11.

Table 11: Average Estradiol Concentration Over 24 Hours (pg/mL) after Treatment with Transdermal and Intranasal Estradiol

Estradiol Dosage Form	Average Concentration over 24 Hours (pg/mL)
transdermal, Vivelle® 50 μg/day patch	34.4
transdermal, Vivelle-dot® 50 μg/day patch	36.8
intranasal, 350 μg/spray	37.8

[00170] Although the invention has been described with respect to particular embodiments, it will be apparent to those skilled in the art that various changes and modifications can be made without departing from the invention.

IT IS CLAIMED:

1. A method of treating benign gynecological disorders in a patient population composed of premenopausal women having no history of endometrial hyperplasia, and who are not receiving exogenously supplied progesterone on a regular or periodic basis, comprising:

administering a formulation comprising a gonadotropin releasing hormone (GnRH) compound, in an amount effective to suppress ovarian estrogen and progesterone production, and an estrogenic compound along with, optionally, an androgen, in an amount effective to prevent signs and symptoms of estrogen deficiency and androgen deficiency over an extended time period.

2. The method according to claim 1, wherein said administering comprises administering by daily intranasal administration, over an extended period of time between about 6 and 12 months, wherein said GnRH compound is administered in an amount effective to suppress ovarian estrogen and progesterone production, and the estrogenic compound is administered in an amount effective to prevent signs and symptoms of estrogen deficiency.

3. The method according to claim 1 or 2, wherein the GnRH compound is a GnRH peptide agonist, and the compound is administered intranasally.

4. The method according to any preceding claim, wherein the GnRH compound is selected from the group consisting of deslorelin, leuprolide, nafarelin, goserelin, decapeptyl, buserelin, histrelin, gonadorelin, abarelix, cetrorelix, azaline B, degarelix, and analogs thereof.

5. The method according to any preceding claim, wherein said administering further includes administering an androgen in an amount sufficient to prevent signs and symptoms of androgen deficiency.

6. method according to claim 5, wherein said androgen is testosterone and said administering comprises transdermally administering said testosterone at a daily dose sufficient to increase serum testosterone to the premenopausal range of between 15-80 ng/dL.

7. The method according to claim 5, wherein said androgen is testosterone and said administering comprises intranasally administering said testosterone at a daily dosage of between 0.15-1 mg.

8. The method according to any preceding claim, wherein the GnRH compound is administered by an intranasal route, and the estrogenic compound is 17ß-estradiol administered by a transdermal route, at a daily dose between 0.025 and 0.1 mg.

9. The method according to any preceding claim, wherein said formulation is an aqueous formulation.

10. The method according to any one of claims 1-7 or 9, wherein the GnRH compound and the estrogenic compound are co-administered intranasally, in an aerosol spray containing a daily spray volume between 30 and 200 µL.

11. The method according to claim 10, wherein the spray volume administered includes between 0.15 mg and 0.6 mg of the estrogenic compound, wherein said compound is 17ß-estradiol.

12. The method according to any one of claims 10 to 11, wherein said spray volume further includes 2-hydroxypropyl-ß-cyclodextrin, in a mole ratio of cyclodextrin to total steroid of between 1:1 and 3:1.

13. The method according to any one of claims 1 to 8, wherein said formulation is an aerosolizable dry powder.

14. The method of claim 13, wherein the dry powder also includes testosterone, in a mole ratio of estrogenic compound: testosterone of between 1:1 and 1:2.

15. A nasal spray formulation, comprising an aqueous medium having dissolved therein:

(i) a GnRH compound; and

(ii) an estrogenic compound present in the form of a water-soluble complex with a water-soluble cyclodextrin;

where the concentration of GnRH compound and estrogenic compound in the

formulation are effective, when administered intranasally, to suppress ovarian estrogen and progesterone production and to prevent signs and symptoms of estrogen deficiency, without a significant increase in the risk of endometrial hyperplasia.

16. The formulation according to claim 15, wherein said formulation when intranasally administered as a daily bolus (i) is effective to achieve an average serum concentration over 24 hours of the estrogenic compound that is within 10% of the average serum concentration over 24 hours of the estrogenic compound when delivered transdermally and (ii) is as effective in preventing bone mineral density loss as transdermal administration of the estrogenic compound.

17. The formulation according to claim 15 or 16, wherein the cyclodextrin is 2-hydroxypropyl-ß-cyclodextrin.

18. The formulation according to claim 17, wherein the 2-hydroxypropyl-ß-cyclodextrin is at a concentration between 50 and 300 mg/mL.

19. The formulation according to claim 17 or 18, wherein the 2-hydroxypropyl-ß-cyclodextrin has a degree of substitution between 2 and 8.

20. The formulation according to any one of claims 15 to 19, wherein the GnRH compound is a GnRH peptide agonist.

21. The formulation according to claim 20, wherein the GnRH compound is selected from the group consisting of deslorelin, leuprolide, nafarelin, goserelin, decapeptyl, buserelin, histrelin, gonadorelin, abarelix, cetrorelix, azaline B, degarelix, and analogs thereof.

22. The formulation according to claim 21, wherein the GnRH compound is deslorelin, at a dose between 0.025 and 1.5 mg.

23. The formulation according to any one of claims 15 to 22, wherein the estrogenic compound is 17-ß-estradiol, at a dose between 0.15 and 0.6 mg.

24. The formulation according to any one of claims 15 to 23, which further

includes testosterone as a second or third steroid in the form of a water-soluble complex with the cyclodextrin, and at a dose of between 0.15 and 1 mg.

25. The formulation according to any one of claims 15 to 24, which further includes a progestin as a second or third steroid in the form of a water-soluble complex with the cyclodextrin.

26. The formulation according to claim 24 or 25, wherein the estrogenic compound and the second or third steroid have a combined molar occupancy with respect to the cyclodextrin that is greater than the molar occupancy achievable with either steroid alone.

27. An intranasal drug-delivery system for use in female contraception or in the treatment of benign gynecological disorders, comprising:

a nebulizer operable to deliver a selected volume; and

contained in the nebulizer, a liquid formulation composed of (i) a liquid carrier, (ii) a GnRH compound capable of suppressing ovarian estrogen and progesterone production, and (iii) an estrogenic compound capable of preventing signs and symptoms of estrogen deficiency when co-administered with the GnRH compound;

where the concentration of GnRH compound and estrogenic compound in the formulation are effective, when administered intranasally, to suppress ovarian function and to prevent signs and symptoms of estrogen deficiency, without a significant increase in the risk of endometrial hyperplasia.

28. The intranasal drug-delivery system according to claim 27, wherein said nebulizer is operable to deliver a selected volume between 30 and 200 μL of the liquid formulation in the form of a liquid-droplet aerosol.

29. The intranasal drug-delivery system according to claim 27 or 28, wherein said liquid formulation further comprises a water-soluble cyclodextrin present in the form of a water-soluble complex with the estrogenic compound.

30. The intranasal drug-delivery system according to claim 29, wherein the cyclodextrin is 2-hydroxypropyl-ß-cyclodextrin, at a concentration between 50 and 300 mg/mL.

31. The intranasal drug-delivery system according to claim 30, wherein the 2-hydroxypropyl-ß-cyclodextrin has a degree of substitution between 2 and 8.

32. The intranasal drug-delivery system according to any one of claims 27 to 31, wherein the GnRH compound is deslorelin, at a dose between 0.025 and 1.5 mg.

33. The intranasal drug-delivery system according to any one of claims 27 to 32, wherein the estrogenic compound is 17-ß-estradiol, at a dose between 0.15 and 0.6 mg.

34. The intranasal drug-delivery system according to any one of claims 29 to 33, further including testosterone as a second or third steroid in the form of a water-soluble complex with the cyclodextrin, and at a dose of between 0.15 and 1 mg.

35. The intranasal drug-delivery system according to any one of claims 29 to 34, further including a progestin as a second or third steroid in the form of a water-soluble complex with the cyclodextrin.

36. The intranasal drug-delivery system according to claim 34 or 35, wherein the estrogenic compound and the second or third steroid have a combined molar occupancy with respect to the cyclodextrin that is greater than the molar occupancy achievable with either steroid alone.

37. The intranasal drug-delivery system according to any one of claims 29 to 36, wherein the cyclodextrin is 2-hydroxypropyl-ß-cyclodextrin having a degree of substitution between 2 and 8, and a concentration between 50 and 300 mg/mL.

38. The intranasal drug-delivery system according to any one of claims 27 to 37, wherein the GnRH compound is a GnRH peptide agonist.

39. The intranasal drug-delivery system according to claim 38, wherein the GnRH compound is selected from the group consisting of deslorelin, leuprolide, nafarelin, goserelin, decapeptyl, buserelin, histrelin, gonadorelin, abarelix, cetrorelix, azaline B, and degarelix, and analogs thereof.

40. An improvement in a method for contraception, for treatment of benign

gynecological disorders, or for hormone replacement, by administration of an estrogenic compound and an androgenic compound and, optionally, a progestin compound, said improvement comprising:

administering intranasally a formulation comprised of the estrogenic compound and the androgenic compound complexed with a cyclodextrin;

whereby said intranasal administering is effective to achieve a physiologic effect comparable to that produced when the estrogenic compound and the androgenic compound are administered transdermally.

41. The method according to claim 40, wherein the cyclodextrin is 2-hydroxypropyl-ß-cyclodextrin.

42. The method according to claim 41, wherein the 2-hydroxypropyl-ß-cyclodextrin has a degree of substitution between 2 and 8.

43. The method according to claim 41 or 42, wherein the concentration of 2-hydroxypropyl-ß-cyclodextrin in the formulation is between 50 mg/mL to 300 mg/mL.

44. The method according to any one of claims 40 to 43, wherein the estrogenic compound and the androgenic compound have a combined molar occupancy with respect to the cyclodextrin that is greater than the molar occupancy achievable with either steroid alone.

45. The method according to claim 44, wherein the combined molar occupancy of the steroids is greater than 40%.

46. The method according to claim 44 or 45, wherein the combined molar occupancy of the steroids is greater than 50%.

47. The method according to any one of claims 40 to 46, wherein the estrogenic compound is 17ß-estradiol at a daily dose between 0.15 mg and 0.6 mg and the androgenic compound is testosterone at a daily dose between 0.15 mg and 1 mg.

48. The method according to claim 47, wherein the mole ratio of 17ß-estradiol to testosterone is between 1:1 and 1:5.

49. The method according to claim 47 or 48, wherein the molar occupancies of 17ß-estradiol and testosterone are greater than 20% and 40%, respectively.

50. The method according to any one of claims 40 to 49, wherein said formulation further comprises a progestin compound as a third steroid.

51. An intranasal drug-delivery system for use in contraception, in treatment of benign gynecological disorders, or in hormone replacement, by administration of an estrogenic compound and an androgenic compound, and an optional progestin compound, comprising:

(a) a nasal-spray nebulizer; and

(b) contained in the nebulizer, a formulation comprising an estrogenic compound and an androgenic compound, said compounds in a solubilized form complexed with a cyclodextrin, where the amount of the compounds administered intranasally is such as to produce a biological effect comparable to that produced when the estrogenic compound and the androgenic compound are administered transdermally.

52. The system according to claim 51, wherein the cyclodextrin is 2-hydroxypropyl-ß-cyclodextrin.

53. The system according to claim 52, wherein the 2-hydroxypropyl-ß-cyclodextrin has a degree of substitution between 2 and 8.

54. The system according to claim 52 or 53, wherein the concentration of 2-hydroxypropyl-ß-cyclodextrin in the formulation is between 50 to 300 mg/mL.

55. The system according to any one of claims 51 to 54, wherein the estrogenic compound and the androgenic compound have a combined molar occupancy with respect to the cyclodextrin that is greater than the molar occupancy achievable with either steroid alone.

56. The system according to any one of claims 51 to 55, wherein the estrogenic compound is 17ß-estradiol at a daily dose between 0.15 mg and 0.6 mg and the androgenic compound is testosterone at a daily dose between 0.15 mg and 1.0 mg.

57. The system according to claim 56, wherein the mole ratio of 17-ß estradiol to testosterone is between 1:1 and 1:5.

58. The system according to any one of claims 51 to 57, wherein said formulation further comprises a progestin compound as a third steroid.

59. The system according to any one of claims 51 to 58, wherein said nebulizer is effective to deliver a spray volume of between about 30 µL to 200 µL

60. A method of formulating two or more different steroids in a water-soluble form suitable for uptake by a human subject through mucosal tissue, comprising:

forming an aqueous solution of a cyclodextrin;

adding the steroids to the solution in amounts effective to achieve a combined molar occupancy of the two or more steroids which is greater than the molar occupancy achievable with any of the steroids alone.

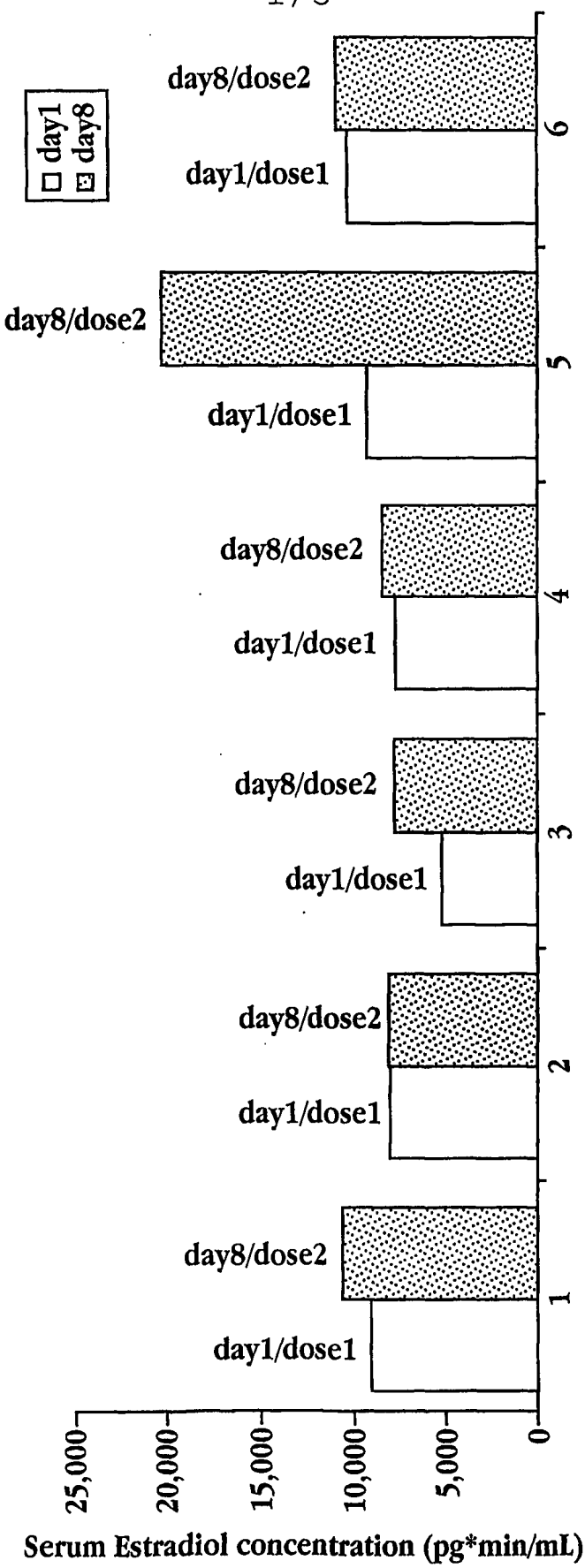
61. The method according to claim 60, further comprising, prior to said adding, heating the aqueous solution of cyclodextrin to above about 70°C.

62. The method according to claim 60 or 61, wherein said adding includes adding the first steroid to the solution until a maximum or near-maximum molar occupancy is reached, then adding the second steroid until a combined maximum or near-maximum molar occupancy is reached.

63. The method according to any one of claims 60 to 62, wherein the cyclodextrin is 2-hydroxypropyl-ß-cyclodextrin and said two or more steroids are an estrogenic compound and an androgenic compound.

64. The method according to any one of claims 60 to 63, wherein said estrogenic compound is 17ß-estradiol and said androgenic compound is testosterone.

65. The method according to any one of claims 60 to 64, wherein said two or more steroids further comprises a progestin compound as a third steroid.


day1
day8
day8/dose2
day1/dose1
6
5
4
3
2
1
Patent No.
25,000
20,000
15,000
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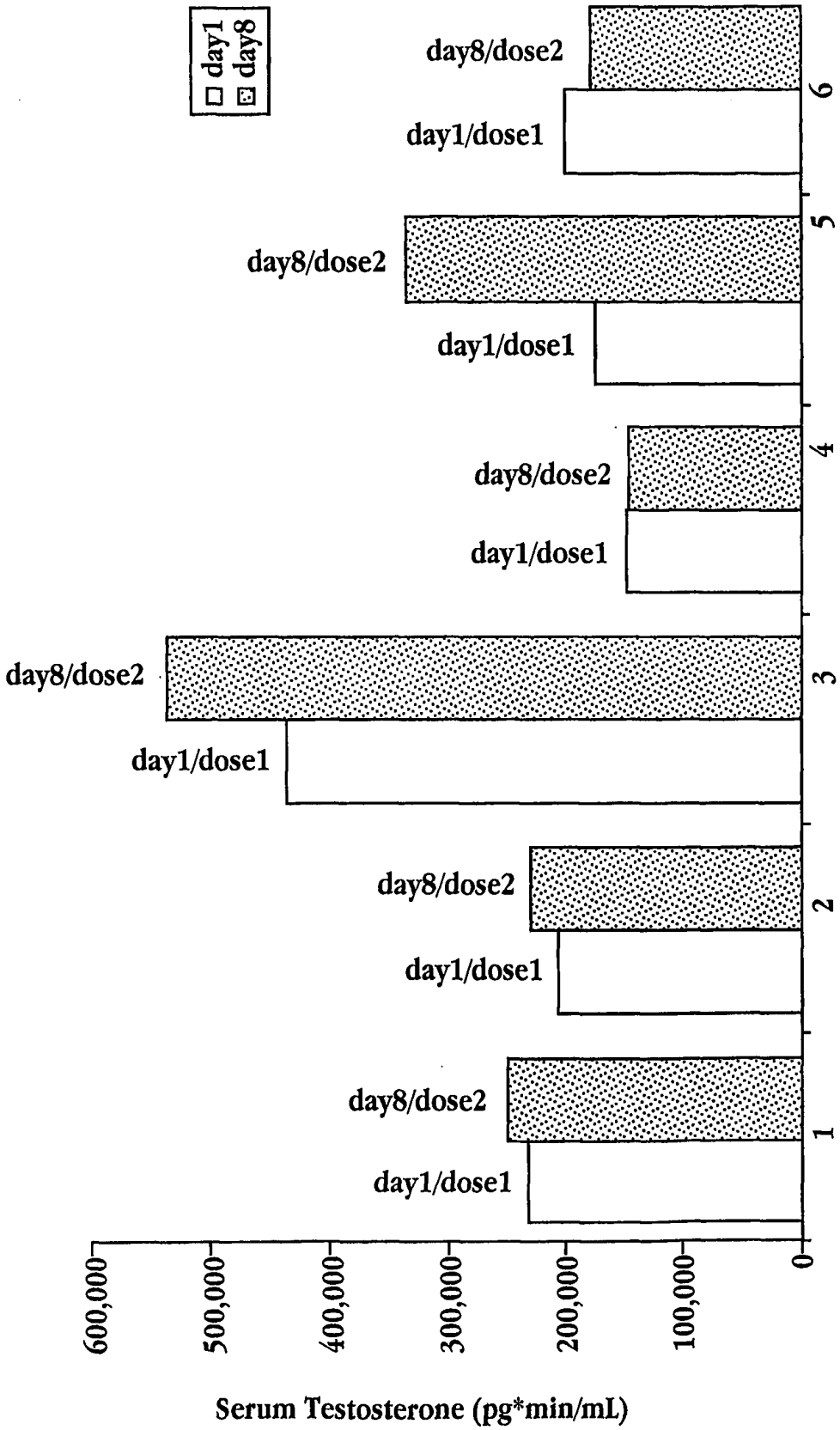
0
Serum Estradiol concentration (pg*min/mL)

Fig. 1A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5
4
3
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500,000
600,000
Serum Testosterone (pg*min/mL)

Fig. 1B

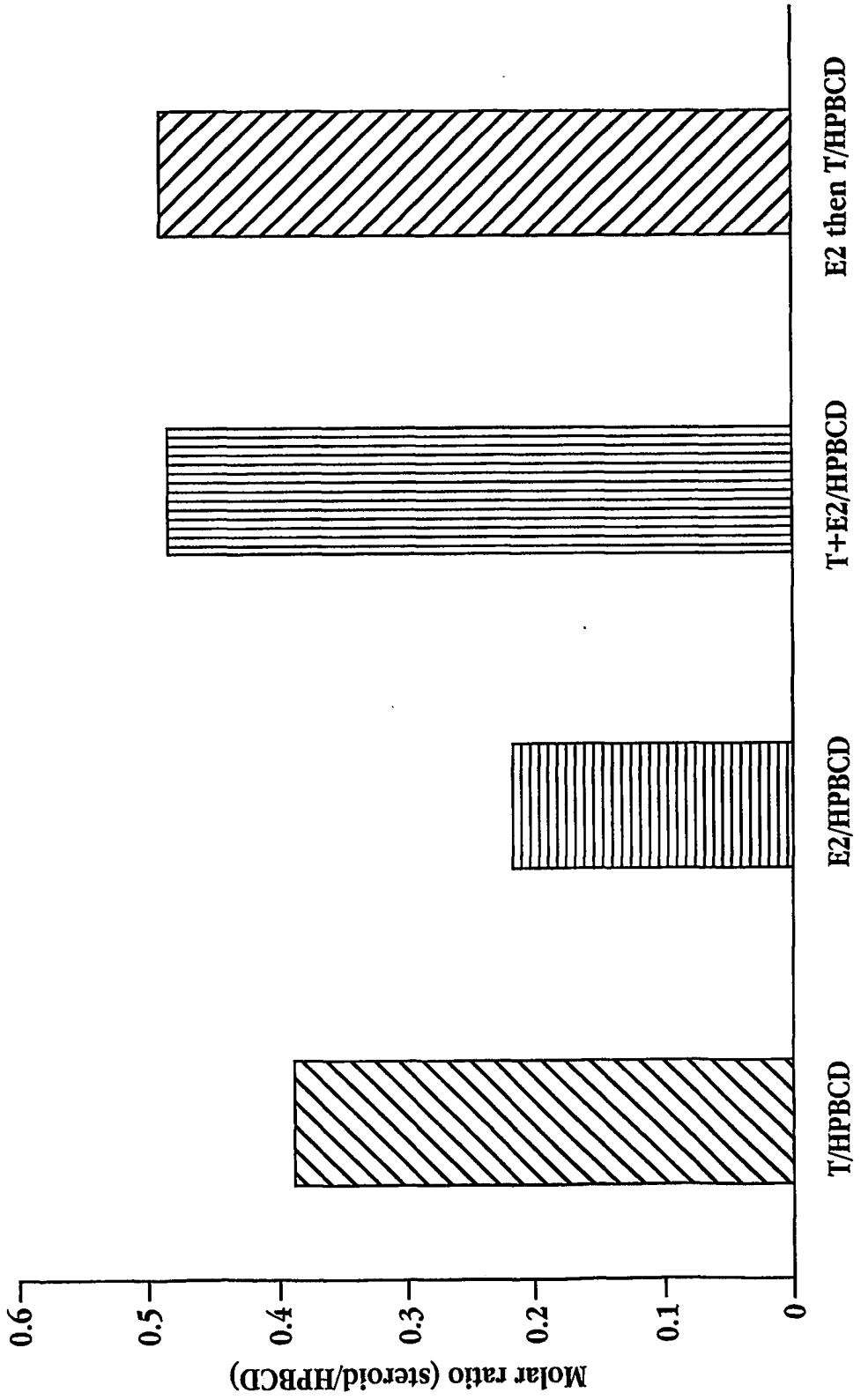
Molar ratio (steroid/HPBCD)
0.6
0.5
0.4
0.3
0.2
0.1
0
T/HPBCD
E2/HPBCD
T+E2/HPBCD
E2 then T/HPBCD
Steroid

Fig. 2

SEQUENCE LISTING

<110> Balance Pharmaceuticals, Inc.
Daniels, AnnaMarie
Daniels, John
Pike, Malcolm
Spicer, Darcy

<120> Methods and Compositions for Treating Benign Gynecological Disorders, Contraception, and Hormone Replacement

<130> 389318001W00

<140> Not Yet Assigned
<141> Filed Herewith

<150> US 60/400,626
<151> 2002-08-02

<150> US 60/400,575
<151> 2002-08-02

<150> US 60/400,576
<151> 2002-08-02

<150> US 10/298,851
<151> 2002-11-15

<150> US 10/298,378
<151> 2002-11-15

<150> US 10/295,337
<151> 2002-11-15

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1 5 10

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1 5 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/24337

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 9/14
US CL : 424/43, 45, 46
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/43, 45, 46

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, CAS ONLINE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,089,482 A (HERMENS et al.) 18 Feburary 1992 (18.02.1992), see entire document.	1-3, 15-18, 27-31, 40-43, 51-54, 60-62
Y	US 5,116,817 A (ANIK) 26 May 1992 (26.05.1992), see entire document.	1-3, 15-18, 27-31, 40-43, 51-54, 60-62
Y	US 4,596,795 A (PITHA) 24 June 1986 (24.06.1986), see entire document.	1-3, 15-18, 27-31, 40-43, 51-54, 60-62
Y	US 4,476,116 A (ANIK) 9 October 1984 (09.10.1984), see entire document.	1-3, 15-18, 27-31, 40-43, 51-54, 60-62

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
24 September 2003 (24.09.2003)

Date of mailing of the international search report
14 JAN 2004

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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/24337

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claim Nos.: 4-14, 19-26, 32-39, 44-50, 55-59, 63-65
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.